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Modelling the Increase in Variance of Fish Weight

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Arnason, A. N., M. H. Papst, and G. E. Hopky. 1992. Modelling the increase in variance of fish weight. Can. J. Fish. Aquat. Sci. 49: 2–16.

A simple model is described that is very effective in accounting for the variance in fish weights in groups of fish grown under controlled environmental conditions. The model explains the increase in variation over time in terms of final weight and the time required to reach this weight and is independent of initial weight and feeding rate. Results from experiments with rainbow trout (*Oncorhynchus mykiss*) and Arctic char (*Salvelinus alpinus*) were used to confirm the model's fit and to demonstrate how temperature effects can be incorporated. We discuss the use of the model for prediction and for testing for genetic or environmental effects on variation and for quantifying such effects on variance over time.

Les auteurs décrivent un modèle simple qui s'avère très efficace pour décrire la variance du poids de poissons de groupes d'individus élevés dans des conditions environnementales contrôlées. Le modèle permet d'expliquer, indépendamment du poids initial et du taux d'alimentation, l'accroissement temporel de la variance en fonction du poids définitif et du temps nécessaire à l'atteinte de ce poids. Les résultats d'essais sur des truites arc-en-ciel (*Oncorhynchus mykiss*) et des ombles chevaliers (*Salvelinus alpinus*) ont servi à confirmer l'ajustement du modèle et à démontrer comment il était aussi possible d'y inclure les effets de la température. Les auteurs traitent de l'utilisation du modèle pour la prévision et la vérification d'effets génétiques ou environnementaux sur la variance et la quantification de ces effets sur la variance en fonction du temps.

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A great deal of effort has gone into modelling the growth of fish. Recent modelling papers include From and Rasmussen (1984) and Hewett and Johnson (1987), and these papers and Ricker (1979) include reviews of important earlier models. These models have been quite successful in accounting for the main determinants of growth rate: temperature, food, and current weight. They have been shown to apply to many species and food types, provided secondary growth determinants, such as density and water quality, are not limiting. The models tend to be used for two main purposes which we can call prediction and data reduction. In the former, the models are used to predict growth of fish under controlled or known environmental conditions, usually for purposes of stock management. In the latter, the models are used to reduce large numbers of observations over time, either on individual fish weights or on mean weights of batches of fish, to a few parameters describing the overall growth rate under prescribed conditions. This then facilitates comparison of growth under differing condition sets of temperature, food type, feeding rate, genetic strain or species, density, culture system, etc.

In both types of applications, the models have usually been deterministic. When used for prediction, they describe the mean weight but not the variability in fish weights about that mean. When used for data reduction, variation about the model is only used as the error variation for testing among treatments (condition sets). It is difficult to relate error variation to the variation in fish weight itself because most of the data used for fitting growth models have traditionally been batch mean weights and/or transformations of these means (e.g. to specific growth rates). It is unusual, and more expensive, to obtain variance data because this requires either extensive replication or the measurement of individual fish weights within the batch. Even when extensive variation data are available, it is difficult to known how to summarize them so they are of use for prediction or data reduction.

Throughout this paper we shall use the term variation-in-weight to refer to the sample or expected variation in the weights of a batch of fish reared under common environmental conditions. When a batch is followed through time and periodically sampled or censused, this process is referred to as a trial. Our aim is to develop a *variance model* (not to be confused with an analysis of variance model) which explains the expected change in variance over time in response to known, controlled or uncontrolled, environmental influences. Thus a variance model attempts to explain the change in variation-in-weight just as a growth model attempts to explain the change in the mean weight.

There are several reasons why it is desirable to have a variance model. Added to deterministic growth models, it permits prediction of fish size distribution (Sparre 1976; Arnason and Scuse 1986) and the exploration of more realistic management strategies, including the need for, and effects of, grading. A deterministic model may predict when a batch of fish will reach a given mean target weight but not the fraction that can be harvested. A variance model would permit more realistic and efficient production planning and marketing.

In genetic and fish culture experiments, a variance model may permit reduction of data so that more comprehensive or meaningful comparisons of variance-in-weight can be made among strains or culture conditions. For example, suppose one wishes to compare two treatments such as two genetic strains or two feeding rates for their effect on growth rate and variance. At the end of a fixed-length growth experiment, the fish in the two treatment groups will usually exhibit different variances, but they will also probably have different means even if the groups began with near identical means and variances. Thus the experimenter cannot tell to what extent differences in final variances are due to the treatment as opposed to the differences in final mean weight. One way to resolve this is to grow out the slower growing treatment group until its final mean weight reaches that of the other group. Comparisons are now unconfounded by differences in final mean weight, but this is a difficult and inefficient way to resolve the problem and, in any case, cannot solve the problem of possible confounding with differences in initial variances among groups. Purdom (1974) reviewed the need, in genetic selection experiments, to make comparisons of variance that are unconfounded by differences in mean weight and suggested the use of the coefficient of variance: CV^2, the square of the coefficient of variation, CV = (standard deviation/mean). The investigations in this paper will show that, in general, CV^2 does not remove this confounding. The most effective means of removing such confounding is through the use of analysis of covariance (ANCOVA) with the confounding factors as covariates. This permits the adjustment of group effects to account for among-group differences in these factors. However, the tests and estimates in such an ANCOVA are sensitive to whether the form of dependence of the response variable (final variance) on the covariates is correctly modelled. A variance model can tell us what this form of dependence is and how to exploit it to develop an appropriate ANCOVA of the data that is efficient, unbiased, and interpretable.

Both these applications of a variance model have direct relevance to the needs of aquaculturists. Commercial aquaculture requires the marketing of fish of near uniform size. Variation in weights among fish within production lots results in inefficiencies and increased operating costs. Environmental, behavioural, and genetic factors are all thought to play a role in promoting heterogeneity in growth in aquaculture systems. There is increasing interest in understanding how these factors contribute to variability and so there is a need to test for and quantify the effects of these factors on variation throughout the growth cycle, independent of their effect on growth. Understanding the sources of variation can lead to the development of genetic stocks and culture practices that minimize variation. What cannot be eliminated needs to be predicted so that rational management plans can be developed.

In this paper, we develop a mathematical form for a variance model that explains the increase in variance-in-weight exhibited by batches of fish grown under constant conditions. We describe how the model can be fitted; that is, how to estimate the parameters of the model using data on the mean and variance in fish weight recorded at periodic repeated censuses of the batches. We then give the results of fitting this model over a wide range of conditions sets (species, strain, temperature, feeding rate, initial mean- and variance-in-weight) and show that a very simple model satisfactorily encompasses them all. A large part of this investigation is devoted to examining the extent to which the model is insensitive to environmental or initial condition effects such as temperature, feeding rate, and initial mean weight. We also show how to test for and quantify these and other treatment effects while making full use of the data but avoiding the confounding effects caused by differences in initial variance and final mean weight. Finally, we discuss the implications of the model for prediction and data reduction and discuss its application to assessing stock performance in experimental and commercial aquaculture.

Model Derivation and Fitting Procedures

Our model is a generalization of one proposed by Sparre (1976). He developed a stochastic model for growth of a group of fish, all assumed to have the identical initial weight and grown together for a period of time, t, under constant conditions of temperature, T, and feedlevel, fl. He defined fl as the fraction of maximum ration where the latter ($fl = 1$) is the minimum feeding rate at the current temperature and mean weight that produces maximum growth. Under these conditions, Sparre's model can be summarized as follows.

Fish weights at time t are normally distributed with mean μ_t and variance σ_t^2 where μ_t is some function of t, given μ_0, fl, and T, and the variance is

$$(1) \quad \mu_t^2 = \alpha^2 t^2 \mu_t^2.$$

The form of the growth function for μ_t is based on Ursin (1967), but its exact form does not need to concern us here. The single parameter, α, of this variance model specifies the rate of increase in the CV per unit time. The remarkable feature of this equation is that it does not directly involve the growth conditions, T and fl, or the initial size, μ_0, but predicts the final variance-in-weight solely in terms of the final mean weight, μ_t, and the time it took to achieve it, provided the batch of fish has zero variance at time 0. T, fl, and μ_0 will, or course, determine μ_t, but once this is determined, the values of T, fl, and μ_0 can be discarded for purposes of determining σ_t. An implication of this is that two different sets of growth conditions that produce the same final mean weight over a given interval (e.g. high T and low fl, or high fl and low T) will produce the same variance. By extension, one would also expect the model to hold even if T and fl were not constant over the interval $[0, t]$. This model's simplicity is important for the fitting, use, and interpretation of the model.

Sparre's variance model (1) incorporates some biologically realistic as well as some unrealistic properties. First, because of inclusion of the elapsed time, t, in equation (1), the model predicts that a batch of fish maintained at constant mean weight by manipulation of fl or T or both will continue to increase in variance. Second, the model allows either decreasing or increasing variance at rations below maintenance, depending on how rapidly μ_t decreases with respect to time. Both phenomena are apparent in our experimental data. The unrealistic properties are the initial variance of zero and the prediction that the CV in the batch increases linearly in time ($CV_t = \alpha t$), contrary to most observations on CV in weight or length (Purdom 1974; Papst et al. 1992) over extended periods of time.

We generalised equation (1) to the form

$$(2) \quad \sigma_t^2 = \alpha t^\beta \mu_t^\gamma.$$

This is now a three-parameter model that includes equation (1) as a special case (α, β, and γ replaced by α^2, 2, and 2, respectively), but it allows much more realistic behaviours for the CV. Our general model, like Sparre's, is only succinct and use-

ful to the degree that the parameters α, β, and γ are independent of the conditions, T, fl, and μ_0.

We cannot fit the model to data until we deal with the requirement of zero initial variance. It is not feasible to conduct experiments where all the fish in the batch have identical initial weights. Stochastic simulation studies (Arnason and Scuse 1986, their fig. 1 and 2) show that even a small initial CV of 10 or 20% can contribute substantially to the final variance, and so the initial variance must be measured and adjusted for. In the Appendix, we show how to derive an adjustment for initial variance in fish weights, σ_0^2, given equation (2) and a specific model for mean growth, μ_t. However, to avoid specifying the form of the growth model, we also derive the following adjustment:

$$(3) \quad \sigma_t^2 - \sigma_0^2 = \alpha\, t^{\beta}\, \mu_t^{\gamma}$$

or

$$(4) \quad \log\{\sigma_t^2 - \sigma_0^2\} = \log\{\alpha\} + \beta\log\{t\} + \gamma\log\{\mu_t\}.$$

The data required to fit the model in the form specified by equation (4) can be obtained from growth trials in which fish are individually censused at $N + 1$ sample times t_i, $i = 0,1,\ldots,N$, at which the fish are individually weighed to produce a sample mean weight and variance-in-weight, w_i and s_i^2, both based on n_i fish in the batch at time t_i. Since w_i is an estimate of μ_t and s_i^2 of σ_t^2 when $t = t_i$, we can then fit the model using a weighted multiple linear regression of the form

$$(5) \quad y_i = b_0 + b_1\, Lt_i + b_2\, Lw_i + \epsilon_i$$

where $y_i = \log\{s_i^2 - s_0^2\}$, $Lt_i = \log\{t_i\}$, and $Lw_i = \log\{w_i\}$ and where ϵ_i is the residual error, assumed distributed normally with mean 0 and variance proportional to $1/n_i$. Comparing equation (5) with (4) shows that the coefficients of the fitted model, b_0, b_1, and b_2, provide estimates of $\log\{\alpha\}$, β, and γ, respectively. There are some problems in applying this analysis, largely due to the nonindependence of successive (repeated-measure) weighings of the same group of fish. There are also potential problems of poor parameter identifiability and precision. In the Appendix, we review these problems and how they can be dealt with by appropriate design and analysis of the experiment. The analysis must be able to deal with repeated-measure data because such growth trials are an efficient way for the experimenter to use fish stocks and tanks. It is also desirable to fit the model in its linearized form, since reliable software for providing estimates and tests is widely available and easy to use and interpret. Even within these constraints, however, there are many different ways in which the data might be analysed. In the Appendix, we review some of these alternatives and justify the methods used here.

In order to avoid problems at the analysis stage, the following design criteria should be met. The analysis uses data from repeated-measure individual-fish weighings of several batches of fish where two or more treatment combinations for feeding, temperature, or initial weight are applied at the batch level. At least some of the batches should involve more than one weighing after the initial one. Each treatment is replicated two or more times if batches are small or, if large, independent random subsamples may be taken from a single replication per treatment. Initial CV for each batch should be kept small and weighings should be well spaced in time (at least 2 wk; longer for treatments that produce slow growth) to avoid decreasing variance. Treatments below maintenance will typically not yield much useable data because y cannot be formed when the variance decreases. In any case, as we shall show, the model does not appear to hold in such situations.

Treatment effects on the parameters of the model can be explored by including the treatments as class variables added to the linear model (5), in which Lt and Lw are covariates. For example, a one-factor model might involve three temperature treatments. The treatment main effect tests for an effect of temperature on α. If the $T \times Lt$ and $T \times Lw$ interactions are nonsignificant, there is no perceptible treatment effect on β and γ. After eliminating effect terms from the model, the goodness-of-fit of the reduced model, relative to the full model before eliminating terms, can be assessed by a general linear test (Neter et al. 1985, chap. 3.8). Similar analyses and model reductions can be applied to a two-factor experiment involving, say, three temperature treatments each at two feeding treatments. The repeated-measure structure of the observations can be allowed for in these analyses as described in the Appendix.

The design and analysis considerations described above would need to be applied to each stock separately if interest centres on comparisons among stocks. There is no reason to expect two stocks to share common variance model parameters, just as there is abundant evidence (Baker 1983) that they do not share common growth parameters unless they are closely related.

All analyses were carried out using PROC GLM in SAS (SAS 1985). This procedure also supports weighted fits (the weights used were the n_i) and the residual plots necessary to check the homogeneity of error variance and the overall goodness-of-fit of the model.

Methods

The two experiments analyzed below were conducted at the Rockwood Aquaculture Research Centre of the Freshwater Institute, Department of Fisheries and Oceans. The facility is described in Papst and Hopky (1982). Experiment 1 was designed as a short density-controlled set of trials to permit fitting and comprehensive testing of both the deterministic component (for μ_t) and the variance component of the Sparre (1976) growth model. Methods of fitting the deterministic component are reported elsewhere (Arnason and Bannatyne 1988). Experiment 1 was a complete factorial experiment with three replicates of each combination of the factor levels shown in Table 1: three temperatures (T) $\times$ three feeding-rate levels (f) $\times$ four initial weights (w_0). Methods for setting the factor levels are described below. Each replicate of a factor combination consisted of a 6-wk growth trial in a separate tank. Batch sizes (Table 1) were inversely proportional to w_0 so that all trials began at the same tank density. Two replicates were censused every 14 d, while the third was censused only at the beginning and end of the trial. This was done to test for handling effects of the intermediate censuses on growth rate or variance, but neither effect was detected when the final census data were analyzed using mean- or variance-in-weight at the final census as the y variable and replicate as a treatment effect. The data from all three replicates were therefore included in the analyses which followed. The data from the starvation treatments (f = 0%) were not used in fitting because they produced frequent observations of decreasing variance (47 of 84 observations), while those with $f > 0\%$ produced few (4 of 168). The starvation trial data, and the results of eight additional "heavy-fish" trials (described below), were analyzed to determine if

TABLE 1. Summary of design factors for the two experiments.

Exp.	Stock	Controlled factors	Treaments (levels)
1	Nisqually rainbow trout (many parents)	Genetic strain Initial density ($\approx$10 g·L^{-1}) Food type	T = temperature (7, 10.5, 14°C) f = feeding rate (0, 75, 150% of table values) w_0 = initial mean weight (12, 24, 48, 96 g) Replicates (upper, lower, control) n[a] = 48, 24, 12, 6 TT[b] = 108 NW = 4 (2 for control) DT = 14
2	Nauyuk Lake Arctic char (full sibs)	Genetic family Initial age, Mean (0.66 g), and SD (0.16 g) in weight Initial density ($\approx$10 g·L^{-1}) Food type Temperature (10°C)	f = feeding rate (50, 100, 150%) Replicates (1, 2, 3, 4) n = 75 TT[b] = 12 NW = 10 (13 for f = 50%) DT = 21

[a]Sample size (n) in Experiment 1 depended on initial mean weight.
[b]TT is total trials; NW is number of weighings per trial; DT is average days between weighings.

the fitted variance model (5) can be used to extrapolate results beyond the factor levels used for fitting.

Experiment 2 was designed to test the effect of feeding rate on an ungraded fish family composed of the progeny of a single male/female mating. Twelve growth trials were conducted (four replicates at three feeding-rate levels) using 75 fish per trial (Table 1). The experiment was initiated when the population mean weight was approximately 0.67 g and was terminated when the populations within each treatment (f) reached approximately 20 g. Fish were censused every 21 d.

Description of Rearing System

Each trial for Experiment 1 was carried out in a 0.61-m^2, 60-L fibreglass tank. The tanks were arranged in rows of 24, consisting of 12 top and 12 bottom tanks. Each row was attached to a gravel biological recirculation-filter unit. Tank rows represent a blocking factor with temperature applied at the whole-block level. Each w_0 treatment required nine tanks (three replicates × three f) in each of three rows (T). The "heavy-fish" trials were conducted in four 120-L tanks in each of two rows to permit the following treatments: two replicates × two f (75, 150%) × two T (10.5, 14°C) at w_0 = 190 g. Experiment 2 employed 12 circular fibreglass tanks with an inside diameter of 31.5 cm and containing 20 L of water. All tanks shared a common biological filter.

All tank systems allowed a high degree of temperature control; water temperature varied by no more than 1°C. Oxygen saturation levels remained stable throughout the experiments at approximately 80% saturation. The photoperiod was set at 12 h of light and 12 h of darkness.

In both experiments, fish were hand fed three times daily on a commercial trout feed (Martins Feed, Elmira, Ont., Canada). The treatment levels for feeding (f, in Table 1) are given as percentages of the manufacturer's recommended feeding table values which specify a feed percent (grams of food per 100 g of fish per day) as a function of current temperature and mean weight (Fig. 1). This table value, along with the feeding rate (f), was used after each census to recalculate the daily food fed to each tank. Note that feeding rate (f, expressed as a percent)

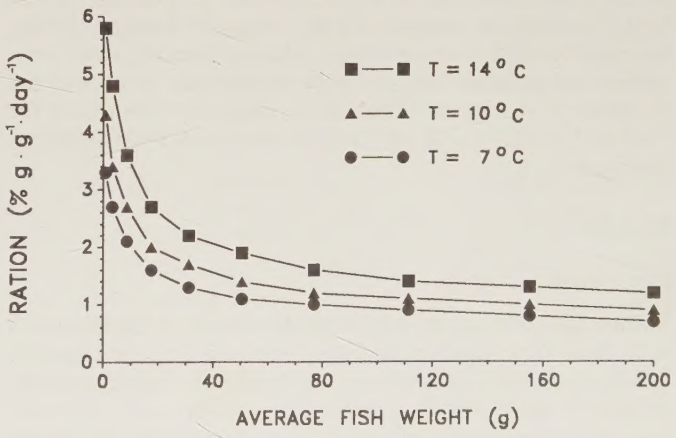

FIG. 1. Feeding table values used to set feed amounts in the growth trial experiments. Tables specify recommended percent feeding (grams of food per 100 g of fish per day) for maximum ration given the current mean weight and temperature. The tables specify constant ration values for weights in the ranges delimited by the cut points 0.2, 1.5, 5, 12, 23, 39, 62, 92, 131, 179, and 221 g. These constant values have been plotted (points) against the midpoints of the range 0.8, 3.3., 8.5, 17.5, 31.5, 50.5, 77, 111.5, 155, and 200 g but can be linearly interpolated as shown (lines). Values shown here are taken as a feeding treatment level (f) of 100% and were scaled appropriately for the desired f (Table 1).

is not the same as Sparre's feedlevel (*fl*, expressed as a proportion).

Fish were censused by anaesthetizing and individually weighing all the fish in a tank. Total tank weights were also determined by weighing all the fish from the tank together. Dead or distressed fish were removed daily, weighed, and frozen. Removals were not replaced and feeding was not adjusted until the next census; removals were never substantial (one or two per trial, at most).

Source of Fish Stocks

Rainbow trout (*Oncorhynchus mykiss*) for Experiment 1 resulted from the 1983 spawning of the Nisqually strain of rainbow trout. This strain was originally received as eyed eggs in 1974 from Nisqually Trout Farms, Washington, USA. The exact origin of the strain is not known. The fish used were from the random matings of several males and females in a single spawning year. Fish were held in different stock tanks that were manipulated for several months prior to commencing the experiment to bring the tank mean weights close to the treatment mean weight, w_0. Fish were then graded out into an initial weight class with a CV of 10% or less. The graded fish were then randomly allocated to tanks for the 27 treatment batches (three T × three f × three replicates) in the required numbers.

Arctic char (*Salvelinus alpinus*) used in Experiment 2 originated from the mating of a single male and female fish from the hatchery's Nauyuk Lake broodstock. The broodstock were originally received in January 1978 as eggs from Nauyuk Lake on the Kent Peninsula, N.W.T., Canada. The biology of the wild population has been described in detail by Johnson (1980). Seventy-five fish were randomly selected from the stock population and assigned to a randomly chosen tank for each of the 12 (three f × four replicates) trials and fish were reared for 3 wk at f = 100% and 10°C before treatments were randomly assigned.

Results

Experiment 1

Our main purpose in presenting the results of Experiment 1 is to show how standard statistical methods for linear models can be used to test for invariance of the model to environmental effects (T and f) and to initial mean and variance. We show how to use graphical and analytical methods to do this, in the process deriving the simplest model that is consistent with all the data. We can then examine how well this simplest model extrapolates to new data and to other predictive uses of the model.

The analysis was carried out in a number of stages, the results of which are summarized in Table 2. Because this is a three-factor experiment (four-factor if we consider replicates as a factor), there is a large number of possible interaction terms. We therefore analyzed each temperature class separately to determine the effects of feeding rate and initial size. Having determined that replicates produce no significant effects, we then carried out a separate factorial ANCOVA for each temperature class. The results for the middle temperature class (10.5°C), shown in Table 2(a), are representative of all three within-temperature-class analyses. In model (a)(i) of Table 2, all the covariate and class variables and their interactions are included. The first two terms ($Lw = \log\{w_t\}$ and $Lt = \log\{t\}$) show the overwhelming importance of the covariates and hence of the parameters β and γ. The next three treatment-effect terms show that there is little effect of feeding rate (f) and inital weight (w_0) on α. The three Lt × treatment interaction terms show that they do not affect β, and the three Lw × treatment terms show that they do not affect γ. This is confirmed by dropping the six covariate × treatment terms to give model (a)(ii) in Table 2. This test provides a more powerful test for treatment effects on α, but again, the last three terms indicate no strong effect. This model provides a somewhat poorer fit (R^2 is reduced from 95 to 91%), but a relative goodness-of-fit test indicates that model (a)(ii) is not significantly poorer than model (a)(i), the reduction in R^2 being largely attributable to fewer model parameters: 9 versus 23 in model (a)(i). Applying the same test with model a (iii) as the reduced model versus model a(8) as the full model shows that model (a)(iii), with no treatment effects on α, β, or γ whatsoever, is just as good in explaining the data as the full model, despite a further reduction in R^2 to 88%.

We can now analyze the combined data across all three temperature treatments, assuming no effects of f and w_0, to determine the effect of temperature (T) in the model. In the first analysis, model (b)(i) of Table 2, there is a very significant effect of temperature on α in addition to the very large effect of the two covariates. There is some evidence that γ is not the same in all temperature classes because of the significant Lw × T interaction. Indeed if the covariate interaction terms are dropped to give model (b)(ii), the model is a significantly worse fit, despite a very small drop in R^2 from 89 to 88%. This is because, with such very large degrees of freedom for these tests, there is enormous power to detect even the slightest differences in γ among temperature classes. The estimates of γ within each temperature class indicate that this is the case, and the plots discussed below also confirm that there is no great danger in assuming γ to be invariant to temperature. This cannot be said for the effect of temperature on α. If the T main effect is dropped, there is a notable drop in R^2, to 84%, and the lack of fit of this model, (b)(iii), relative to (b)(i), is enormously significant. Thus the analysis points to model (b)(ii) as the minimum-parameter variance model that adequately describes these data.

We now examine the estimates for this simplest model and see if any further simplifications are suggested by the estimates. The parameter estimates for this model are shown in 1(a) of Table 3. We note that a change of temperature from 14 to 10.5°C has the same effect on $\log\{\alpha\}$ as a change from 10.5 to 7°C. This suggests a linear response of $\log\{\alpha\}$ to T which was confirmed by a 1-df contrast test of this hypothesis. The estimate of β does not differ significantly from 1. This is not generally true for all model fits, but it does allow us, in this case, to simplify the model further for purposes of plotting and interpretation. The parameter estimates for the model with these two further simplifications are given in 1(b) of Table 3: 168 observations have now been reduced to a three-parameter model that is independent of f and w_0 and yet explains 88% of the total variability in y = log{increase in variance}.

Rearranging the model slightly aids construction of a validity plot. Because $\beta = 1$, the time effect can be incorporated into y to give the following model:

$$(6)\quad y = \log\{(s_t^2 - s_0^2)/t\} = \log\{\text{increase in variance per unit time}\} = \alpha_0 + \alpha_1 T + \gamma \log\{w_t\}$$

where the parameters α_0, α_1, and γ are estimated by the values shown in 1(b) (Table 3). This form of the model makes it

TABLE 2. Results of sequential model fits using ANCOVA on y = log variance increase. Covariates are Lw (log final weight) and Lt (log elapsed time); class variables are f (feeding level), w_0 (initial weight class), and T (temperature). F values for individual terms give stepwise significance (after all terms above it in the table are included in the model) and R^2 gives stepwise percent of total variance in y explained by the term. Results in (a) are for Experiment 1 and T = 10.5°C and are typical of all three within-T analyses. Covariate × class interaction terms have been combined into a single term (e.g. Lw × class is $Lw \times w_0$, $Lw \times f$, and $Lw \times w_0 \times f$ combined). Results for the complete data (all temperatures) of Experiment 1 are in (b). Results for Experiment 2 are in (c). Columns (ii) and (iii) give the results of reducing the model in (i) by eliminating terms.

	(i)				(ii)				(iii)			
Term	R^2	df	F	p	R^2	df	F	p	R^2	df	F	p
(a) Experiment 1: T = 10.5°C												
Lw	79.1	1	449.4	<0.001	79.1	1	393.9	<0.001	79.1	1	257.8	<0.001
Lt	8.6	1	48.6	<0.001	8.6	1	42.6	<0.001	8.6	1	36.2	<0.001
w_0	0.4	3	0.8	0.53	0.4	3	0.7	0.59				
f	0.6	1	3.7	0.07	0.6	1	3.2	0.08				
$w_0 \times f$	2.2	3	4.2	0.01	2.2	3	3.2	0.02				
Lw × class	2.6	7	2.1	0.07								
Lt × class	1.0	7	0.8	0.59								
Overall	94.5	23,31	23.4	<0.001	91.0	9,45	50.3	<0.001	87.7	2,52	185.2	<0.001
Goodness of fit relative to (a)(i)						14,31	1.5	0.19		21,31	1.9	0.57
(b) Experiment 1: All temperatures combined												
Lw	77.8	1	1125.9	<0.001	77.8	1	1004.8	<0.001	77.8	1	776.9	<0.001
Lt	6.0	1	87.2	<0.001	6.0	1	77.8	<0.001	6.0	1	60.1	<0.001
T	3.8	2	27.6	<0.001	3.8	2	24.6	<0.001				
$Lw \times T$	1.1	2	8.2	0.001								
$Lt \times T$	0.5	2	3.4	0.04								
Overall	89.3	8,155	161.4	<0.001	87.7	4,159	282.9	<0.001	83.8	2,161	418.5	<0.001
Goodness of fit relative to (b)(i)						4,155	5.8	<0.001		6,155	13.1	<0.001
(c) Experiment 2: All trials combined												
Lw	97.8	1	9985.1	<0.001	97.8	1	7814.8	<0.001	97.8	1	7750.5	<0.001
Lt	0.6	1	61.6	<0.001	0.6	1	48.2	<0.001				
f	0.2	2	9.1	<0.001	0.2	2	7.1	0.001	0.6	2	25.3	<0.001
$Lw \times f$	0.2	2	9.3	<0.001					0.2	2	5.8	0.004
$Lt \times f$	0.2	2	8.1	0.001								
Overall	99.0	8,107	1262.5	<0.001	98.6	4,111	1969.3	<0.001	98.6	5,110	1562.5	<0.001
Goodness of fit relative to (c)(i)						4,107	8.7	<0.001		3,107	11.6	<0.001

possible to plot the observed and predicted y-values in linear form using meaningful axes (Fig. 2), confirming the accuracy of the model. In particular, the parallelism and significant separation of different temperature lines is easily checked.

A more stringent validity test is provided by using the model to extrapolate to conditions beyond the range of those used to fit the model. This was done by using equation (6), along with the observed values of s_0^2, w_t, T, and t to predict s_t^2, at each census, for the 36 starvation trials and the eight "heavy-fish" trials. The observed and predicted standard deviations (s_t), combined over replicates, were then plotted (Fig. 3 and 4). This gave a less crowded and better-scaled plot than did s_t^2. It is apparent from Fig. 3 that the model (6) severely overpredicts s_t for the starvation trials, particularly for the larger initial weights and higher temperatures. The extrapolation of the model to very high weights (Fig. 4) gives excellent predictions when f is above maintenance levels. The poorly predicted standard deviations in Fig. 4 are all for the trial at f = 75%, T = 14°C; this treatment combination turned out to be close to maintenance, producing a growth rate of less than 0.5% per day over the 42 d, from w_0 = 182 g to w_t = 219 g.

One further validation helps to confirm that the model adequately accounts for temporal and initial-variance effects. All the validations and plots considered so far use the direct variance increase computed over the elapsed time from the beginning of the experiment. The predicted values can also be computed from the stepwise variance increase between each pair of censuses. There are several reasons why comparing these two predictions is a useful validation. First, the stepwise variance increases were not used in the fitting and introduce a new set of initial means and variances; one would expect that the accuracy of such predictions is sensitive to any model misspecification of the role of initial mean and variance. Second, the model may be used for making stepwise predictions (Arnason et al. 1984) and it is important to demonstrate that these will be accurate. There is good reason to be concerned about this problem for, as demonstrated in the Appendix, the model is not theoretically consistent in its predictions made by

TABLE 3. Parameter estimates for the simplest model of variance increase. Symbols are t (elapsed time), w_t (mean weight at time t), and s_t^2 (variance at t). Greek letter coefficients are the parameters estimated, and parentheses, e.g., $\alpha(T)$, $\alpha(f)$, indicate that different parameters are needed for each treatment level. Experiment 1 results are in 1(a) using T(°C) as a class variable and in 1(b) as a continuous covariate and assuming $\beta = 1$. Two models for Experiment 2 seem equally acceptable: 2(a), assuming parallel covariate ($\log(t)$, $\log\{w_t\}$) responses, and 2(b), no temporal (t) covariate and nonparallel $\log\{w_t\}$ response. In all models, the dependent variable is $y = \log\{s_t^2 - s_0^2\}$.

	1(a)			1(b)		
Model	$y = \log\{\alpha(T)\} + \beta\log\{t\} + \gamma\log\{w_t\}$			$y = \alpha_0 + \alpha_1 T + \log\{t\} + \gamma\log\{w_t\}$		
R^2	87.7%			87.6%		
Estimates	Parameter	Est.	SE	Parameter	Est.	SE
	$\log\{\alpha(14)\}$	−6.739	0.364	α_0	−8.556	0.255
	$\log\left\{\frac{\alpha(10.5)}{\alpha(14)}\right\}$	−0.370	0.106	α_1	0.109	0.015
	$\log\left\{\frac{\alpha(7)}{\alpha(14)}\right\}$	−0.759	0.108			
	β	0.895	0.098	β	1.0	—
	γ	1.884	0.070	γ	1.867	0.068
	2(a)			2(b)		
	$y = \log\{\alpha(f)\} + \beta\log\{t\} + \gamma\log\{w_t\}$			$y = \log\{\alpha(f)\} + \gamma(f)\log\{w_t\}$		
	98.7%			98.7%		
	Parameter	Est.	SE	Parameter	Est.	SE
	$\log\{\alpha(150\%)\}$	−6.001	0.532	$\log\{\alpha(150\%)\}$	−4.146	0.101
	$\log\left\{\frac{\alpha(100\%)}{\alpha(150\%)}\right\}$	0.244	0.072	$\log\left\{\frac{\alpha(100\%)}{\alpha(150\%)}\right\}$	−0.007	0.142
	$\log\left\{\frac{\alpha(50\%)}{\alpha(150\%)}\right\}$	0.280	0.088	$\log\left\{\frac{\alpha(50)\%}{\alpha(150)\%}\right\}$	0.549	0.134
	β	0.571	0.168	β	0	—
	γ	2.134	0.127	$\gamma(150\%)$	2.159	0.049
				$\gamma(100\%) - \gamma(150\%)$	0.182	0.072
				$\gamma(50\%) - \gamma(150\%)$	−0.052	0.069

the two methods. In practice, however, the inconsistencies were slight (Fig. 5), although there is a clear tendency for the stepwise predictions to fall below the direct predictions, increasingly so as the percent change in mean weight increases. Both sets of predictions used in Fig. 5 were formed using the parameter estimates obtained by fitting the model to the direct data with the control replicate dropped. The stepwise predictions are computed by using the predicted final variance from the previous census as the initial variance and using the number of days since the previous census as the elapsed time t, in equation (6). As a further test of model consistency, we fit the same model to the stepwise data and it gave virtually identical estimates of the parameters α_1 and γ; β could not be estimated separately from α_0 because t was constant (14 d).

Experiment 2

Experiment 2 involves fewer factors than Experiment 1 because temperature and initial size were kept constant across tanks but it has much larger batch sizes, numbers of replicates, and number of censuses (Table 1). This provides a more stringent test of the ability of the model to reflect variance changes over the long term. It also demonstrates a narrower but typical use of the model to test a specific hypothesis using carefully controlled single-factor experimental data.

As before, we begin by fitting the full model and examining terms to see which complicating factors can be eliminated. Results of ANCOVA based on equation (5) and using f as a treatment class variable are shown in Table 2(c). Replicates were combined in this analysis because there was no significant effect of replicates ($p > 0.01$ for all replicate effects). The Lt covariate accounts for almost all of the variability in y, the log{increase in variance}, while the Lt covariate and the treatment (f) make very small but statistically significant contributions. This model, (c)(i) in Table 2, is biologically unreasonable: the estimates of β for this model was negative (-0.32 ± 0.32) although not significantly different from 0 ($p = 0.31$), implying that fish held near maintenance would be expected to exhibit unchanged or declining variance. Most of the estimates from this model were very imprecise and strongly correlated. The model seems to be overdetermined with Lt and the covariate–treatment interactions ($Lw \times f$, $Lt \times f$) playing much the same role in explaining the tiny remaining variability in y after Lw is accounted for. Dropping the interaction terms leads to model (c)(ii) in Table 2 and to the precise, reasonable estimates shown in 2(a) of Table 3. Dropping the terms involving Lt has the same effect ((c)(iii) in Table 2 and 2(b) in Table 3). Neither reduced model lowers the R^2 much (from 99.0 to 98.6%), but in each case, the reduction is statistically signifi-

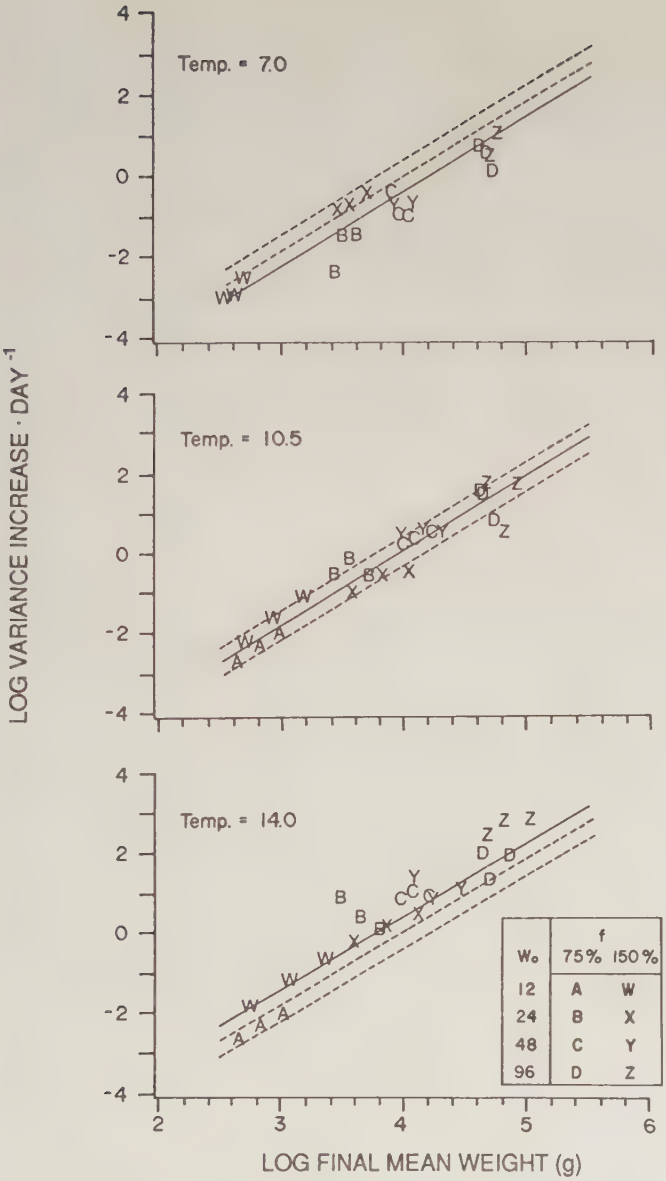

FIG. 2. Observed and predicted log{increase in variance per day} versus log{final mean weight} for Experiment 1. Values are plotted separately for each temperature with observed values given by letters identifying (w_0, f) treatment class (''A'' values are not shown in the top panel, as they were completely overlain by the ''W'' values). Predicted values are computed using model 1(b) of Table 3 and are shown, in each temperature panel, as a solid line. The broken lines show, for comparison, the predicted values at the other two temperatures to demonstrate the magnitude of the temperature effect.

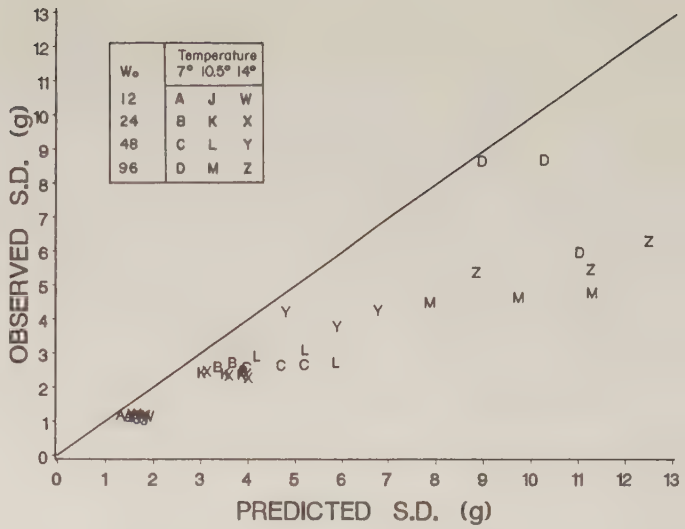

FIG. 3. Observed versus predicted standard deviation (SD) in weight for the starvation (f = 0%) trials. Observed values are given by letters identifying (w_0, T) treatment. Each letter occurs three times, corresponding to the three weighings, and each occurrence is the average SD over the two or three replicates for that weighing and treatment. The solid line is the 45° predicted versus predicted line. The model predicts much larger SD-in-weight in fish held at starvation than is actually observed.

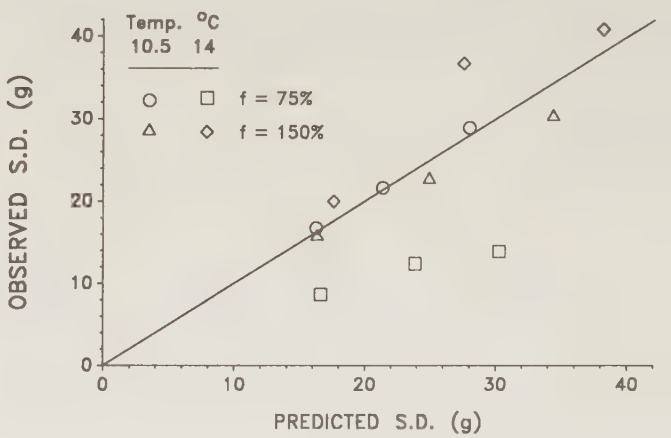

FIG. 4. Observed versus predicted standard deviation (SD) for the ''heavy-fish'' trials. Results are plotted in the same fashion as in Fig. 3, with symbols identifying (f, T) treatment; average SD over two replicates at each of three weighings. The worst predicted set is for the treatment (f = 75%, T = 14°C, plot symbol = □) that produced the least growth.

cant ($p < 0.01$). Nevertheless, some reduction of the overdetermined full model seems warranted; the first reduction is easier to interpret, but the second reduction was somewhat better at predicting the observed response of CV over time.

It is worth examining the meaning of the fitted parameters with reference to the effect of f on CV response. If these reduced models (2(a) and 2(b) in Table 3) are back-transformed to predict $E(\mathrm{CV}_t)$, the expected coefficient of variation at time t, we obtain for model 2(a) of Table 3:

$$(7)\quad E(\mathrm{CV}_t) = ((s_0^2/w_t^2) + \alpha(f)\, t^{\beta}\, w_t^{\gamma-2})^{1/2}$$

and for model 2(b) of Table 3:

$$(8)\quad E(\mathrm{CV}_t) = ((s_0^2/w_t^2) + \alpha(f)\, w_t^{\gamma(f)-2})^{1/2}.$$

The symbols for these equations are defined in Table 3. In both equations, there is a term for an initial variance effect that decays as time and mean weight increase, but it accounts for the noticeable concavity in the CV plot (Fig. 6). Provided there are no interaction effects (as in equation (7) where β and γ are not affected by treatment, f), the coefficients $\alpha(f)$ define the relative rate at which CV^2 or variance increases, once this initial effect wears off, for fish of equivalent weights but at different treatment (f) levels. For example, from 2(a) in Table 3, $\alpha(50\%)/\alpha(150\%) = \exp\{0.280\} = 1.32$ and $\alpha(100\%)/\alpha(150\%) = 1.28$. This means that for fish of comparable weight, the increase in variance-in-weight, per unit time, per unit weight,

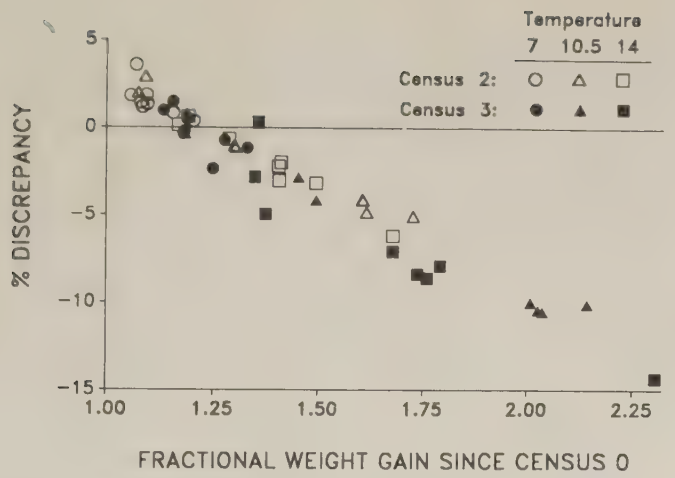

FIG. 5. Discrepancy between stepwise and direct predicted standard deviation in Experiment 1 data. Discrepancy is measured as the difference between the two predictions expressed as a percentage of the latter. The stepwise predictions tend to fall below the direct predictions, but mostly by less than 5% after two steps and less than 10% after three steps. For clarity, only one replicate at each (f, T, w_0) factor combination is plotted.

is around 30% higher for fish fed at 50 or 100% ration, relative to fish fed at 150% ration. These differences seem large, but because variance is in grams squared, they represent only a 5.5% difference in CV or standard deviation. Clearly, the effect of feeding on CV is extremely small but detectable and in the expected direction: lower feeding levels lead to higher rates of gain in variability in weight even after adjustment for differences in weight gain due to ration.

The parameter estimates for the second reduced model (2(b) in Table 3) lead to the same conclusions. The estimated f = 50% feeding effect is $\alpha(50\%)/\alpha(150\%) = \exp\{0.549\} = 1.73$, an 8.6% difference in CV. However, the f = 100% feeding effect is not easily summarized because of nonparallelism (treatment 2 in Fig. 7) but is clearly intermediate between treatments 1 (f = 50%) and 3 (f = 150%) for weights between 1 and 20 g.

Experiment 2 provides a further demonstration of the need for an initial variance term in the model despite the fact that initial mean weight and variance-in-weight did not differ significantly among the 12 tanks. It might then seem unnecessary to adjust for the roughly constant initial variance. If this were so, the analyses could equally well be applied to the variance-in-weight (dependent variable $y = \log\{s_t^2\}$) instead of increase in variance ($y = \log\{s_t^2 - s_0^2\}$). We tried these analyses and found that they also gave excellent fits ($R^2 > 99\%$), but the models were difficult to interpret because of strong replicate effects and many covariate interaction effects; moreover, models of this form cannot reproduce the pronounced concavity in the CV plot, since they always predict that CV increases as mean weight increases. The difficulties in interpretation arise from the nonparallelism evident from the interaction effects. The nonparallelism results because all replicates and treatments share a common origin, so most effects manifest themselves as differences in slope radiating out from this common origin.

Discussion

The variance model proposed in equations (4) and (5) takes the form of a generalized Taylor's power law (Southwood

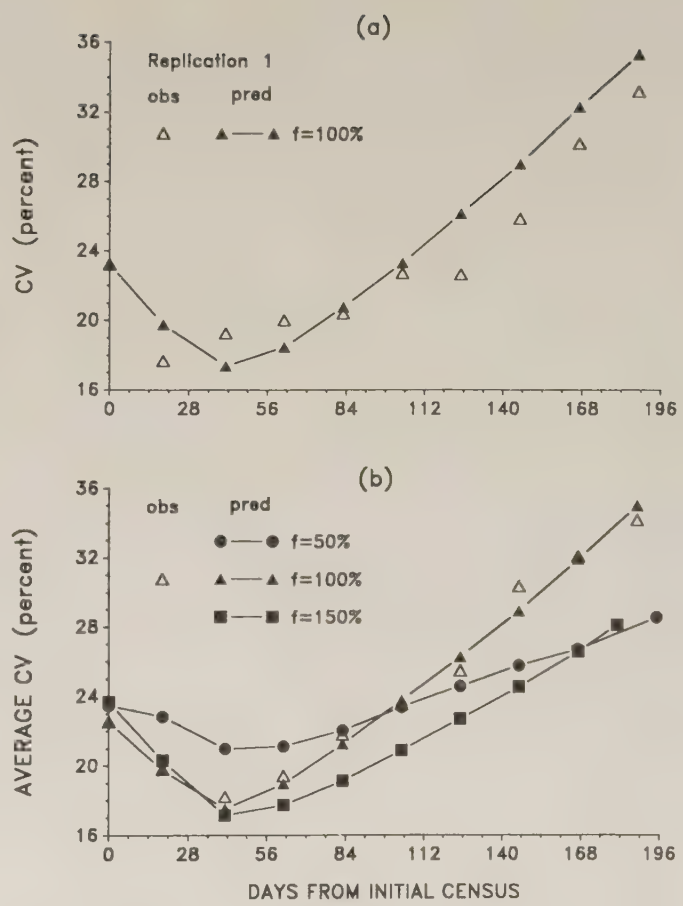

FIG. 6. Experiment 2, observed and predicted CV versus elapsed time. The predicted values are computed using equation (8) and observed values of initial variance (s_0^2) and final mean weight (w_t). (a) One of the four replicates of treatment 2 (f = 100%) is shown to illustrate the high variability and strong serial correlation of individual replicates about the predicted model; (b) observed and predicted values at each weighing (t) are averaged over the four replicates, eliminating both problems and permitting comparisons among treatments. For clarity, observed values are given for only one treatment, but observed means were similarly close to predicted means for the other treatments.

1966). This "law" states that, in expectation, variance is a power function of the mean:

$$E(s^2) = \alpha\mu^{\gamma}.$$

It has been widely observed to hold with abundance and biomass data (Downing 1979). It has also been observed in those few instances where biologists have properly taken account of the variance structure when fitting growth models (Kingsley 1979). It is therefore not surprising that it holds for fish growth data, nor is it unexpected that the model can be generalized to make the coefficients (α, γ) functions of controlled experimental treatments. What is novel here are two key results: (1) sometimes an additional power term, t^{β}, is needed to give adequate fits and predictions and (2) when the response variable is *increase* in variance, rather than variance itself, environmental effects on the model are either negligible or are confined to the scale parameter α. We discuss these two points in turn.

Inclusion of a temporal power term, t^{β}, is not always necessary. It was included in the model for historical reasons, to test if Sparre's use of the term was justified, and for theoretical reasons, to allow for change in variance-in-weight over time

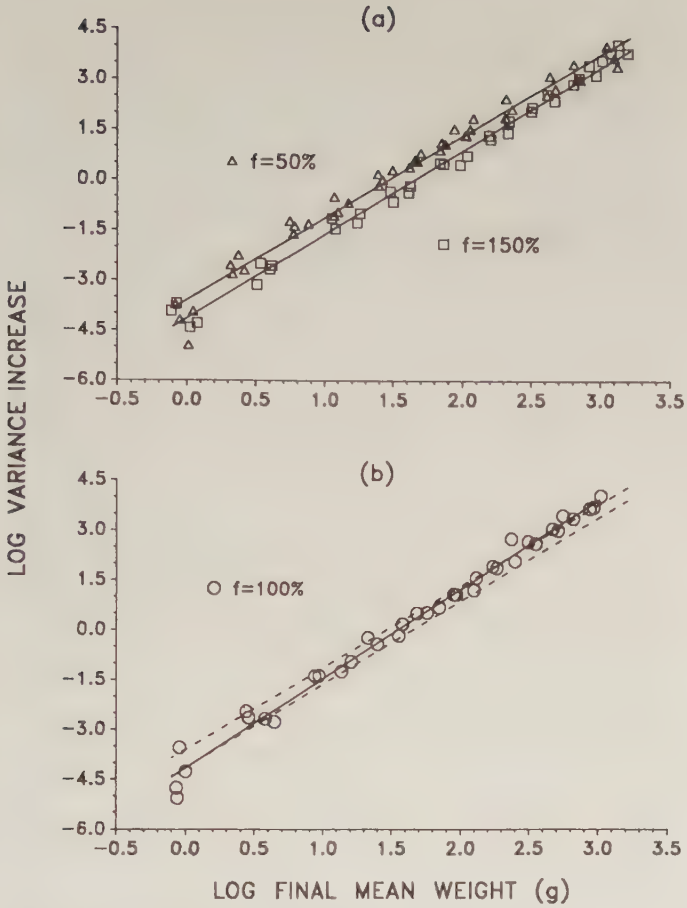

FIG. 7. Experiment 2, observed and predicted log{increase in variance} versus log{final mean weight}. Observed values are given by symbols identifying treatment; predicted values, computed using model 2(b) in Table 3, are shown as lines. (a) Treatments 1 and 3; (b) treatment 2 along with the predicted values (broken lines) for treatments 1 and 3. The (statistically significant) vertical separation between the $f = 50\%$ and the $f = 150\%$ lines means that the high-ration fish gain variance-in-weight at a lower rate than low-ration fish of the same weight.

even when weight is constant. The model, possibly including a temporal term, may hold at low growth rates, but the starvation data presented here (Fig. 3) indicate that the parameters of the model must change sharply as feeding rates approach and fall below the rate required for maintenance. Since we had very little data in the neighbourhood of maintenance feeding rates, the theoretical justification for a temporal term remains unresolved. Nevertheless, the term seems to be empirically justified even though it never makes a large contribution to explaining y, the log{increase in variance}, after the contribution of weight is accounted for. In Experiment 1, the term was necessary to obtain a good predictive model whereas in Experiment 2, the most satisfactory model did not include a temporal term.

Evidence on the significance of both the temporal and the weight parameter, β and γ, was also gained when the variance model was fit to a large number of genetic trials carried out at the Rockwood facility by two graduate students (Tompkins 1989; Wangila 1989). The design and sample sizes of these experiments were similar to those of Experiment 2 (Table 1) and generally involved two replicated temperature treatments applied to a full-sib family fed above-maximum ration ($f = 150\%$). The model was fit to each family separately. The magnitude and significance of the temporal coefficient varied widely among families, significant values tending to fall in the range 0.5–1.5. Estimates of β were generally quite precise and the magnitude of differences among families appeared to reflect their degree of genetic similarity. For many families, particularly of rainbow trout rather than for Arctic char, β did not differ significantly from 0 and the temporal term was not needed in the model. As in Experiments 1 and 2 reported here, Lw accounted for most of the variation in y, and the parameter γ for all families was always around, although generally differing significantly from, 2.0. The estimates of this parameter are always very precise but, for reasons given in the Appendix, can change substantially if the temporal term is dropped from the model. This effect can be seen by comparing the estimates for model 2(a) with those for model 2(b) in Table 3. As with the parameter β, differences in the parameter γ between families tend to be smaller for more closely related families. These results from the genetic trials, along with our findings discussed below, that β and γ do not vary much with temperature or feeding rate, seem to indicate a strong genetic influence with minimal environmental modification of these two parameters.

In this paper, we examined three controlled environmental effects on variance-in-weight: grading (w_0) and temperature in Experiment 1 and feeding rate in Experiments 1 and 2. There is no evidence that grading has any appreciable effect on any of the model parameters, since we found no significant w_0 main effects or interaction effects (Table 2(a)). This means that the adjustment for an arbitrary initial variance by fitting increase in variance completely removes this effect, at least when the initial CV is small (<20%). The adjustment seems to work independently of the initial distribution: in Experiment 1 the initial distribution was roughly uniform as a result of a fixed grading window to establish the initial batch mean weights (e.g. 10–15 g for the $w_0 = 12.5$ g treatment); in Experiment 2 the ungraded equal-aged fish had a roughly normal weight distribution. Data with very large initial variance (CV>20%) and strongly multimodal initial distributions, as might result from a mixture of two or more age groups, are probably not suitable for fitting the variance model. This does not, however, limit the model to predicting variance only for batches of fish that have small initial variance. The distribution that results from growth starting from any arbitrary initial distribution can be predicted using a matrix projection method (Arnason et al. 1984). An accurate variance model that accounts for small initial variance is essential for this purpose, since the matrix method constructs the resulting distribution as the composite of contributions from each of the successive grade windows represented by the initial histogram. Some caution, and perhaps further validation, is necessary before the model is used to predict variances resulting from such poolings. As shown in the Appendix, the model is theoretically inconsistent in predicting the results of pooling samples, just as it was inconsistent in predicting variances computed stepwise. We carried out a limited number of theoretical and empirical studies of this problem. We will not describe them here except to say that, again, the inconsistencies were not large.

Feeding rate also has a very minor effect on the model parameters. In Experiment 1, we found that any feeding effects could be ignored for purposes of prediction, except for feeding rates that produce small or negative growth rates. Some modification of the model would be required to explain change in variance-in-weight at these lower feeding rates, but this might be difficult to do because feeding rate is difficult to quantify in a mean-

ingful way. The most meaningful quantification of feeding rate is feedlevel (Sparre 1976): the amount fed as a fraction of the maximum ration. Maximum ration, defined as the minimum ration above which no increase in growth rate occurs, is specific to the current mean weight and temperature. Precise empirical determination of maximum ration is costly and time-consuming, and manufacturer's tables, purporting to give maximum ration values, are notoriously inaccurate (Jorgensen and Jobling 1989). Fortunately, it does not appear to be necessary to have an accurate quantification of feedlevel as input to the variance model. Feeding rate, whatever it may be, affects the growth rate and hence the final weight w_t, but once w_t is known or predicted, there is no further contribution of feeding rate to the variance model. Note that even in Experiment 2 where feeding effects were the main interests, they were extremely small (less than a 9% increase in CV produced by a threefold decrease in feeding rate) and were only detectable because the experiment was very large in terms of batch size, number of replicates, and number of weighings. In practical aquaculture applications, where feeding is usually near maximum ration, such small effects would have little effect on the validity of predicted variance-in-weight.

Temperature, unlike feeding and grading, has an appreciable effect on rate of increase in variance-in-weight in addition to its effect on growth (w_t). Its effect is primarily on the scale parameter α and not on the temporal or weight coefficients, β and γ. This leads to parallelism of the temperature lines in the log-linear plots against weight. As a consequence, the effect of a temperature difference on variance-in-weight can be quantified independently of current weight, and comparisons between trials can easily be adjusted for temperature differences (within, but not among, genetically distinct stocks) using ANCOVA. The linear response of $\log\{\alpha\}$ to T found in Experiment 1 permits prediction at interpolated T values. Extrapolation to extreme temperatures may be dangerous. The temperature range over which linearity was observed is wide (7–14°C) but lies entirely below the temperature for optimum growth rate (15–16°C for rainbow trout). Experience with growth model parameters (From and Rasmussen 1984; Papst et al. 1982) suggests that nonlinearity in temperature response may become strongly evident at temperatures above the optimum.

We believe that the model proposed here is more appropriate and insightful for examining changes in variance-in-weight than any analysis based on the change in CV with time. The log-linear form of our model makes it easy to estimate the parameters and validate the fit. Parallelism of lines corresponding to different treatments on the same genetic stock will usually result because environmental influences, if not negligible, seem generally to be confined to the parameter α and are thus easy to test for and quantify. Comparisons between treatments are equivalent to tests of effects on α and are independent of any treatment effect on weight gain. Comparisons between stocks grown under fixed environmental conditions are also straightforward. If $\beta = 0$ or does not differ between stocks, then comparisons are particularly succinct: a stock with larger γ shows a greater rate of gain in variance per unit increase in weight, while a larger α shows a higher rate of gain in response to the given environment. Log-linear plots similar to Fig. 2 ($\beta \neq 0$) and 7 ($\beta = 0$) also provide useful summaries, although parallelism is not to be expected when comparing stocks.

None of these succinct, unambiguous comparisons is possible by examining plots of CV_t versus t (or by examining CV_t^2 versus t, as recommended by Purdom 1974). This is because differences between treatments in CV are confounded by treatment effects on growth rate and are also obscured by initial variance effects and very high variability and serial correlation in CV_t. Moreover, the plots are typically nonlinear and may be nonmonotonic (e.g. Fig. 6) making it difficult to model CV directly. To illustrate how confounding can lead to misinterpretation, consider what would be observed if our model is correct and there is a treatment effect on growth rate but none on variance. For simplicity, we assume that there are two treatments and $\beta = 0$. In expectation, then, each treatment will show a CV plot of the form

$$E(CV_t) = ((s_0^2/w_t^2) + \alpha\, w_t^{\gamma-2})^{1/2}.$$

The initial variance effect (inner parentheses) can cause the CV curves to cross as the result of small treatment differences in initial variance and growth rate. Even after this effect decays as w_t becomes large, the CV curves will diverge. If $\gamma > 2$, then a treatment that produces a higher growth rate will have a higher CV at any given time. If $\gamma < 2$, then the treatment producing higher growth rate will have a lower CV at any given time. Yet in fact, there is no treatment effect in the sense that when fish reach the same final weight under each of the two treatments, they will exhibit identical CV. The times at which they reach this common final weight will differ between treatments because of the different growth rates. Since most aquaculture is directed at growth to a given target weight, we contend that the appropriate conclusion here is that there is no treatment effect on variance-in-weight. The question of times to reach a given target weight can be studied separately by fitting an appropriate growth model. The differences in the CV plot are, in this case, entirely due to the differences in growth rate and are not due to differences in the variance mechanism between treatments. Only if $\gamma = 2$ (exactly) will the CV plots converge, and then only after the initial effects decay.

Similar confounding problems can occur when there is a treatment effect on both growth rate and variance-in-weight. For example, a stock that is both faster growing and subject to lower rates of gain in variance and so is more desirable for aquaculture might, in fact, show a higher CV at a given time than another, less desirable stock. This is formally possible when γ is less than 2. More likely, the CV plots for the two stocks would cross and recross, due to high variability and initial effects, leading one falsely to conclude that there was no difference in variability. The use of our model and analysis method instead would permit unlinking the gain in variability from the gain in weight, even though in nature, the two processes occur simultaneously and at different rates among different stocks or in different environments.

We believe that our variance model has very wide applicability and utility for prediction and data reduction beyond the controlled hatchery experiments described in this paper. The model could easily be applied to periodic census data from pond or cage cultures, although subsampling would be more practical than complete censusing. Such data are more prone to producing biased means and variances, so steps must be taken to develop and test for an accurate sampling technique. Feeding rates can be highly variable over time and feedlevels may be unknown without affecting the fit or predictive ability of the model because feeding would typically be near maximum ration and at these levels the model is insensitive to feeding rate. Uncontrolled temperature variation also poses no serious problem: β and γ are independent of T and the fitted model should

estimate an average α close to that appropriate for the overall time-average temperature. Model predictions would be reasonably accurate if applied to similar temperature profiles but not if, say, a model derived from high-temperature (summer) data were applied to low-temperature (fall) situations. Care must then be taken to plan data gathering over a whole production cycle and possibly using separately fitted models for production cycles initiated at different seasons of the year. Estimating the temperature response of α explicitly (as in Experiment 1) might be more difficult using production data with uncontrolled T. Nevertheless, the descriptive power of the model for comparing rates of gain in variance-in-weight under different treatments (stock, food type, etc.) would still apply to production data, provided treatments were carried out simultaneously and subject to the same temperature variations.

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Appendix

Derivation of a Correction Factor for Initial Variance

Sparre recognized that if a proposed model for variance in w_t assumes that the variance in w_0 is 0, then one must find a way of "correcting for the initial standard deviation being not zero" (Sparre 1976, appendix A4). Although Sparre did fit his variance model to (very limited) data, he did not reveal how the correction was done, nor did he give direct evidence of goodness of fit for his estimated variance model. The form the correction must take is easily written down using the formula for unconditional variance. First, we denote the conditional mean, given w_0 (exactly), using a tilde, i.e.

$$E[w_t|w_0] = \mu(t, w_0) = \tilde{\mu}_t.$$

Equation (2) then gives the conditional variance:

$$\mathrm{VAR}[w_t|w_0] = \sigma^2(t, \mu(t, w_0)) = \alpha\, t^{\beta}\, \tilde{\mu}_t^{\gamma}.$$

If we now allow w_0 to be a random variable with mean μ_0 and variance σ_0^2, then the unconditional variance at time t is

$$(A.1) \quad \sigma_t^2 = \mathrm{VAR}[w_t] = E[\sigma^2(t, \mu(t, w_0))] + \mathrm{VAR}_{w_0}[\mu(t, w_0)].$$

The first (expectation) term of (A.1) represents the variance that would result after time t if $w_0 = \mu_0$ and can be approximated by $\alpha\, t^{\beta}\, \mu_t^{\gamma}$ where μ_t is the unconditional mean:

$$\mu_t = \underset{w_0}{E}[\mu(t,w_0)] \approx \mu(t,\mathrm{E}[w_0]) = \mu(t,\mu_0).$$

The second (VAR) term of (A.1) gives the correction term due to variation in w_0, but it cannot be evaluated in general; a specific form for the growth model $\mu(t,w_0)$ must be assumed. For example, if we assume exponential growth at (instantaneous) rate r:

$$\mu(t,w_0) = w_0\exp\{rt\} = \tilde{\mu}_t,$$

then

$$\mathrm{E}[\mu(t,w_0)] = \mu_0\exp\{rt\} = \mu_t$$

and

(A.2) $$\mathrm{VAR}[\mu(t,w_0)] = (\exp\{rt\})^2\mathrm{VAR}[w_0] = (\mu_t^2/\mu_0^2)\,\sigma_0^2.$$

After substituting these into (A.1) and rearranging, we obtain

$$\mathrm{CV}_t^2 - \mathrm{CV}_0^2 = \alpha\, t^{\beta}\, \mu_t^{\gamma-2}$$

where $\mathrm{CV}_t = \sigma_t/\mu_t$. Thus the power model for conditional variance (equation 2) plus the exponential growth model imply that the corrected model, accounting for initial variance, should be a power model explaining the *increase in* CV^2. When we applied this model to our data, using $y = \log\{\text{increase in } \mathrm{CV}^2\}$, we found that it gave extremely poor fits: much more of the data had to be discarded because of declines in CV and there were very strong replicate, treatment, and interaction effects. The correction factor derived above is incorrect because fish growth is not exponential (despite the frequent use of estimates of r as a measure of growth rate). With this growth model, the predicted growth trajectories starting at two initial values separated by a small distance d in w_0 (i.e. $\mu(t,w_0)$ and $\mu(t,w_0 + d)$) diverge exponentially. This leads to a correction factor (A.2) that amplifies the effect of σ_0 as the mean weight μ_t increases. More accurate models (Ursin 1967; Sparre 1976) are all characterized by instantaneous growth rates that decline as μ_t increases (Jobling 1983), leading to very slow or negligible divergence in $\mu(t,w_0)$ from $\mu(t,w_0 + d)$, at least over small ranges of t and d. Unfortunately, the analytic complexity of these models makes it impossible to evaluate the algebraic form of the correction factor. However, if we assume no divergence, i.e. $\mu(t,w_0 + d) = d + \mu(t, w_0)$, then $\mathrm{VAR}[\mu(t,w_0)]$ is independent of t and hence equals $\mathrm{VAR}[\mu(0,w_0)] = \sigma_0^2$. Using this correction factor in (A.1) leads to the model used in this paper as given by equation (3). This probably somewhat underestimates the correction factor, leading to some bias in the parameters, but the bias cannot be serious or we would have found large treatment effects. This would have been particularly evident in Experiment 1 where the initial variance was strongly correlated with the initial weight treatment, w_0.

The two correction factors represent the extremes: the true correction factor must lie somewhere in between, but evidently is closer to the latter one based on nondivergence. However, this model cannot be entirely correct because it can be shown that the model displays theoretical inconsistencies in ways that bear directly on how the model might be used for predictions. We give a mathematical demonstration that these inconsistencies exist in the model and then comment on our investigations into the extent to which they were evident in our experimental data.

The first inconsistency can be termed a time-interval inconsistency: the model does not always give the same prediction for σ_t^2 given σ_0^2 and μ_t whether applied directly over a single time interval, or stepwise over subintervals. For example, if $t = 3$, we could obtain σ_3^2 in a direct single step as $\sigma_0^2 + \alpha 3^{\beta}\mu_3^{\gamma}$ or stepwise by obtaining $\sigma_1^2 - \sigma_0^2 = \alpha\mu_1^{\gamma}$, $\sigma_2^2 - \sigma_1^2 = \alpha\,\mu_2^{\gamma}$, and $\sigma_3^2 - \sigma_2^2 = \alpha\mu_3^{\gamma}$. The intermediate parameters (μ_1, σ_1^2, μ_2, σ_2^2) can be eliminated using $\sigma_2^2 - \sigma_0^2 = \alpha 2^{\beta}\mu_2^{\gamma}$ and $\sigma_3^2 - \alpha_1^2 = \alpha 2^{\beta}\mu_3^{\gamma}$. It can then be shown that only $\beta = 0$ or 1 gives the same prediction for σ_3^2. By using other time intervals, it can be shown that no nonzero value of β always gives a consistent model of the form adopted here. If we denote the direct and stepwise computed variances at time 2 by σ_2^2 and ρ_2^2, respectively, then the theoretical discrepancy can be derived as

$$(\rho_2^2 - \sigma_2^2)/\sigma_2^2 = [(\mu_1/\mu_2)^{\gamma} - (2^{\beta} - 1)]/[\sigma_0^2/(\alpha\mu_2^{\gamma}) + 2^{\beta}].$$

This explains why the discrepancy in Fig. 5 becomes increasingly negative as the fractional weight gain increases. It also shows that the discrepancy will be less if σ_0^2 is increased.

The second inconsistency can be called pooling inconsistency. Suppose two replicates of the same sample size have similar but not identical initial means μ_0 and ν_0 and initial variances σ_0^2 and ρ_0^2. If they are grown under common conditions so that a common model applies to both, we get at time t: $\sigma_t^2 - \sigma_0^2 = \alpha\, t^{\beta}\, \mu_t^{\gamma}$ and $\rho_t^2 - \rho_0^2 = \alpha\, t^{\beta}\, \nu_t^{\gamma}$. If the samples are pooled, then the pooled mean, PM_t, and variance. PV_t, at any time t are computable independent of any model, as

(A.3) $$\mathrm{PM}_t = (\mu_t + \nu_t)/2$$

and

(A.4) $$\mathrm{PV}_t = [(\sigma_t^2 + \rho_t^2)/2] + [(\mu_t - \nu_t)/2]^2.$$

The model predicts the increase in variance of the pooled sample using t and the pooled mean at t, so we must have

(A.5) $$\mathrm{PV}_t - \mathrm{PV}_0 = \alpha\, t^{\beta}\, \mathrm{PM}_t^{\gamma}.$$

The individual sample variance equations can be used to eliminate terms in σ and ρ from this last equation giving

$$[(\mu_t - \nu_t)/2]^2 - [(\mu_0 - \nu_0)/2]^2 = \alpha\, t^{\beta}\,\{[(\mu_t + \nu_t)/2]^{\gamma} - (\mu_t^{\gamma} + \nu_t^{\gamma})/2\}.$$

An assumption of the development of our correction factor was that mean weights do not diverge. Thus the left-hand side of this equation is 0 and the right-hand side will only be 0 if γ is 0 or 1; since neither of these values is even close to values supported by our data, it is clear that the model cannot predict the variance for the individual replicates as well as for the result of their pooling.

Both of these inconsistencies occur in precisely those sorts of situations which occur when the model is used as part of a general model for predicting the distribution of fish sizes from an arbitrary initial distribution and under changing environmental conditions (Arnason and Scuse 1986; Sparre 1976). If the inconsistencies are large in practice, it could invalidate the use of the model for these purposes. The dangers they pose to uses of the model for data reduction are less apparent; they indicate that at least part of what is estimated by the parameters is the degree of failure of the model.

To determine how great the dangers were in practice, we did thorough checks using the data from both Experiments 1 and 2. The check for time-interval consistency is described in the body of the paper and its result in Experiment 1 is summarized

in Fig. 5. The same check done on each of the individual replicates of Experiment 2 showed even smaller discrepancies although the same tendency for the stepwise method to underpredict was evident. To check for pooling consistency, we used pairs of replicates from Experiment 2 and obtained the results of pooling using the observed and predicted variances (equation (A.4) using the observed variances and again using the directly predicted variances within each replicate; observed means were used in both cases). We compared both of these with the predicted pooled results (equation (A.5) using the observed initial pooled variance and the observed pooled means). The discrepancies were very small and will not be reported here. It may be that the inconsistencies are not large with these data because we confined our checks to ones that could be done using the observed means. When means are also predicted at the same time as the variances, the discrepancies may be compounded.

These inconsistencies may be resolvable if a more mathematically rigorous approach to model building is taken. Some attempts have been made using Markov processes (Sukhanov 1980; DeAngelis and Huston 1987), but these all model the mean process simultaneous with the size distribution process, and the models are not analytically solvable for the distribution of its variance unless strongly simplifying assumptions are made. Even then, the question of fitting to data is rarely addressed. DeAngelis and Huston (1987) developed the distribution for the exponential growth model discussed at the beginning of this Appendix under similar assumptions to ours, but their motivation (understanding the length structure dynamics of natural populations) was very different from ours. A more empirical model like ours that permits modelling and analysis of the change in variance without the need to model mean growth is more serviceable to the needs of aquaculture. Until the theoretical inconsistencies of the model are fully understood, care must always be taken when the model is manipulated in ways that are very different from those used in its fitting.

Linear Model Analysis of the Variance-in-Weight Data

The log-linear form of the variance model (equation (4)) led us to recommend a weighted multiple linear regression fit based on equation (5). We discuss here the rationale for this and explain some problems and cautions concerning the analysis.

1. Nonhomogeneous error variance

The usual assumption of linear regression is that the residual error variance, var(ϵ), is constant. The log transformation stabilizes the residual variance when batch sizes, n_i, are constant. This follows by the application of Taylor's power law (Southwood 1966), since VAR[s^2] is proportional to $(E[s^2])^2$ for normally distributed data (Kendall and Stewart 1963, p. 244). Unequal n_i can then be allowed for by weighting each y_i by n_i, thus allowing for more general experiments where the batch size changes, either because of deaths or removals, or deliberate control of the size of different batches, or through the use of subsampling of large batches. In any case, the homogeneity assumption and the effectiveness of the weighting should be checked by weighted residual plots.

The extension of the multiple linear regression model to permit testing of treatment effects involves the use of ANCOVA. These tests should be carried out using a repeated-measures model (Freund and Littell 1981, section 3.3.10). This allows treatments that are applied at the "batch" (tank or trial) level to be tested against the "among-batches" error which is typically larger than the "among-times" error. If we denote these error terms as ERR_b and ERR_t, then replicates and treatment effects (w_0, f, $w_0 \times f$, etc.) are tested against ERR_b, while covariates and treatment × covariate interactions are tested against ERR_t. Similarly, a two-level repeated-measures analysis can be used to take account of treatments that are applied at the whole-plot level (sets of batches, e.g. T) We found, however, that it made no difference to our conclusions whether a repeated-measures or a completely randomized model was used, and all the results reported here are from the completely randomized analysis. These models have a single error term ERR_e, whose expected mean square is the weighted average of those for ERR_b and ERR_t (the weights are the degrees of freedom, df):

$$EMS_e = (df_b\ EMS_b + df_t\ EMS_t)/(df_b + df_t).$$

Since, typically, df_b is much smaller than df_t, EMS_e is numerically only slightly larger than EMS_t but it can be very much smaller than EMS_b. Thus, tests of treatment effects using ERR_e are overly sensitive, giving higher statistical significance than they would have if using ERR_b. This is on the safe side when no treatment effects are expected. The other extreme, a very conservative test that gives lower significance, can be obtained by Box's df correction (Milliken and Johnson 1984, chap. 26). In any case, most of our conclusions are ultimately based on the parameter estimates and their standard errors and we found that these were virtually identical in both analyses.

2. Biased variances s_i^2

Deaths or removals which are nonrandom with respect to size will seriously bias the variances and lead to poor and biased fits. This is particularly so for deaths where the smallest or largest fishes in a batch are most at risk. Purdom (1974) criticized the variance results of Mollander for this reason. The problem can only be avoided by careful experimental practice and avoidance of extreme conditions of temperature, density, etc.

3. Decreasing variances and correlated errors

If $s_i^2 < s_0^2$, then y_i (equation (5)) cannot be formed. If this happens for many data points, the estimates will be seriously biased. It is most likely for small elapsed times t_i and/or sample sizes n_i, and under conditions of slow growth. A reasonable guideline is to aim for $n_i > 30$ and to allow sufficient time to the first census for the percent change in mean weight to exceed the initial percent CV. Hence, it is also desirable to reduce the initial CV by grading by age or size. Nevertheless, repeated-measures y_i residuals will be positively correlated. This problem can be partially solved by a first-difference model: in this case, by regressing $\log\{s_i^2 - s^2_{i-1}\}$ on $\log\{t_i - t_{i-1}\}$ and $\log\{w_{t_i}\}$. Now all the censuses must be widely spaced to avoid decreases in variance, making for rather lengthy experiments. Moreover, if all censuses are equally spaced, then $t = t_i - t_{i-1}$ is a constant and β cannot be estimated. A more practical solution is to fit the undifferenced model and reduce the effect of serial correlation by replication (three or four batches reared with identical stock under identical conditions and censused on the same days). This is feasible when batches must be kept small (<100 fish), but with very large batches, the same end can be achieved by taking subsamples, say of 50–100 fish, at each census.

Other analyses can overcome the problems of decreasing variance and correlated errors but were considered unnecessary or

unsuitable. Nonlinear iteratively reweighted model fitting (Green 1984) based on equation (3) would permit inclusion of decreasing variance-in-weight data, but modelling the correlated errors would still be difficult and model selection and testing of individual terms would be computationally complex. Profile analysis (Srivastava and Carter 1983) is a multivariate technique, commonly applied to repeated-measures growth data, that reduces serial correlation by differencing between time points, but it is difficult to apply if censusing is not at identical times in all batches. Moreover, it obscures the underlying model form and limits the hypotheses that can be tested. Other multivariate approaches are discussed in Ware (1985) and Milliken and Johnson (1984, chap. 31). The latter authors pointed out that these approaches have low power relative to other procedures (for repeated-measures data) and hence, although giving less biased tests, would be even less likely than the analyses used here to find significant treatment effects. The (univariate) repeated-measures analyses discussed in Section 1 above will be exactly correct if the correlation in "among-times" errors satisfies the Huynh–Feldt condition (Milliken and Johnson 1984, chap. 26), but it is unlikely that this condition is met for variance-in-weight data.

4. Confounding of μ_t *and* t

$Lw = \log\{w_t\}$ and $Lt = \log\{t\}$ are strongly correlated. This leads to bias in the estimates and the test of hypotheses (e.g. that $\gamma = 0$). To see the nature of this bias, suppose that model (3) holds with $\alpha = 1$, $\beta = 1$, and $\gamma = 2$ and that $\mu_t = \mu_0 t^{0.5}$ ($t = (\mu_t/\mu_0)^2$). The model then reduces to

$$\sigma_t^2 - \sigma_0^2 = \alpha\mu_0 t^{1.5}$$

or

$$\sigma_t^2 - \sigma_0^2 = (\alpha/\mu_0^2)\mu_t^4.$$

Depending on the order in which the *Lw* and *Lt* terms are fitted, we will either estimate β as 1.5 and find no significant effect of *Lw* or estimate γ as 4 and find no significant effect of *Lt*. Note in both cases that the bias in the estimate corresponding to the first term fitted is upward and in the second is downward (to 0 in this case) because of the positive correlation and that the estimate of α is also biased and no longer invariant to initial weight. To obtain unbiased estimates of α, β, and γ, the experiment must include batches reared under two or more treatments (f, T, w_0) such that it takes different lengths of time to reach any given mean weight. The parameters become separately identifiable and their estimates less correlated the stronger these differences are among treatments. Multiple treatments are also necessary to establish if the parameters are effectively independent of treatment.

Influence of Dissolved Organic Carbon, pH, and Microbial Respiration Rates on Mercury Methylation and Demethylation in Lake Water

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Effects of changes in DOC concentrations, pH, and microbial respiration rates on specific rates of mercury methylation and demethylation in lake water were studied using radioisotopic techniques. Increased concentrations of DOC resulted in decreased specific rates of net methylation, possibly as a result of complexation of inorganic mercury with DOC. A reduction in pH from 7.0 to 5.0 had the greatest effect, causing large to moderate increases in net methylation rate at both low and high DOC concentrations (500–2600 μM). Rates of respiration (indicative of general rates of microbial activity), which were insensitive to pH change over the range tested (5.0–7.0), had the smallest effect on net methyl mercury production rates. We propose the following explanations for three situations in which high mercury concentrations are commonly found in fish. (1) in acidified dilute clear-water lakes, high fish mercury concentrations may be a result of enhanced in-lake methylation; (2) in brown-water circumneutral lakes, where in-lake methylation is inhibited by high DOC concentrations, terrestrial inputs of methyl mercury may be most important; and (3) in brown-water, low-pH lakes, both in-lake and terrestrial sources of methyl mercury may contribute to elevated mercury concentrations in fish.

Des techniques radio-isotopiques ont été utilisées pour l'étude des effets de la modification des concentrations du COD, du pH et des taux respiratoires microbiens sur la méthylation et la déméthylation du mercure en milieu lacustre. L'accroissement des concentrations de COD a donné lieu à une baisse des taux spécifiques de la méthylation nette, probablement à cause de la formation de complexes entre le mercure inorganique et le COD. Une baisse du pH, de 7,0 à 5,0, a permis d'obtenir le plus important effet, soit une augmentation, d'importante à moyenne, du taux de méthylation nette, ceci aux concentrations de COD faibles et élevées (500–2 600 μM). Les taux de respiration, indicatifs de l'activité microbienne générale, qui n'ont pas réagi dans la gamme de pH de l'essai (5,0–7,0) sont le facteur qui a eu le moins d'effet sur les taux de production nets de méthyl mercure. Les auteurs proposent une explication pour les trois situations où des concentrations élevées de mercure sont généralement notées chez le poisson : 1) dans les lacs à eau limpide diluée acidifiée, les concentrations élevées de mercure chez le poisson s'expliquent par un accroissement de la méthylation dans le lac même; 2) dans les lacs presque neutres à eau brune, où la méthylation est inhibée par les fortes concentrations de COD, ce sont les apports terrestres de méthyl mercure qui sont les plus importants; et 3) dans les lacs de faible pH à eau brune, les concentrations élevées de mercure chez le poisson s'expliquent par des apports de méthyl mercure de sources lacustres et terrestres.

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Because essentially all of the mercury in freshwater fish tissue is methyl mercury (99%, Grieb et al. 1990; >95%, Surma-Aho et al. 1986), it is important to understand the factors that control its production and bioaccumulation in aquatic environments. This understanding is presently incomplete. For example, low pH is correlated with high mercury levels in fish tissue (≥0.5 ppm) in both drainage lakes (Wren and MacCrimmon 1983; Hakanson et al. 1988; Grieb et al. 1990) and seepage lakes (Cope et al. 1990; Grieb et al. 1990). In addition, high mercury concentrations in fish also occur in drainage lakes at both low and high pH levels that have high dissolved organic carbon (DOC) concentrations (as Pt–Co colour: Mannio et al. 1986; Minnesota Pollution Control Agency 1985; Paasivirta et al. 1983). However, even though this positive correlation of fish mercury with DOC is seen in drainage lakes, a negative correlation with DOC is found in seepage lakes (Grieb et al. 1990).

These seemingly inconsistent relationships in fish methyl mercury concentrations might be explained partly by differ-

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TABLE 1. Brief description of the water and sediment trap material sources used in all experiments.

Source	Z_{max} (m)	pH[a]	Alkalinity (μequiv·L^{-1})[a]	μM DOC	Used for:
Lake 239	30	7.2	165.2	500–580	Experiments 1, 2, and 3
Lake 302N	12	5.3	2.3	500–580	Sediment trap material[b]
Sphagnum bog	—	~4.0	−182.1	>2000	DOC concentrate

[a]Mean surface water values for May–September 1988.
[b]Collected from the hypolimnion.

ences in factors that control in-lake mercury methylation. For example, the fact that methylation rates and the ratio of methylation to demethylation in Precambrian Shield lakes increase with decreasing pH in the water column and surface sediments (Xun et al. 1987) may explain high fish mercury concentrations in many clear-water acidic lakes (Winfrey and Rudd 1990). However, the situation appears to be more complicated in high-DOC lakes where DOC as well as pH may affect the rate of methyl mercury production.

DOC might affect in-lake rates of mercury methylation and demethylation in at least two ways. Firstly, some forms of terrestrial DOC are known sources of decomposable carbon for microbial populations (Tranvik 1988). Inputs of such organic carbon to lakes may stimulate microbial activity and mercury methylation in a manner analogous to that caused by decomposition of carbon in flooded soils and vegetation (Hecky et al. 1987). Secondly, inorganic mercury is bound strongly by DOC, notably humic substances (Kerndorff and Schnitzer 1980; Lodenius et al. 1987; Jackson 1989). This binding may reduce the bioavailability of inorganic mercury to the microbial methylators, decreasing net rates of methyl mercury production.

The purpose of our study was to provide insight into three factors that may interact and control the production of methyl mercury within lakes. The first set of experiments was designed to examine the influence of DOC concentration on mercury methylation and demethylation at natural pH and constant respiration rates. For these experiments, because increasing the DOC concentration stimulated respiration rates, respiration was held constant by overwhelming the decomposition of the natural DOC with addition of another natural substrate (sediment trap material). The objective of the second set of experiments was to observe the influence of different DOC concentrations on specific methylation and demethylation rates, at a uniform pH of 6.0. The third set of experiments examined the effect of changes in both pH and DOC on specific methylation, demethylation, and respiration rates.

Materials and Methods

Study Site

All experiments and sampling were done during the spring and summer of 1988 at the Experimental Lakes Area (ELA), northwestern Ontario, Canada. The area is located in the Precambrian Shield (boreal forest) and has much exposed granite bedrock with minimal soil coverage. The lakes in the area are typically oligotrophic and of low buffering capacity (Brunskill and Schindler 1971). Water samples were taken mainly from Lake 239, a lake that has not been manipulated experimentally. The sample sources are briefly described in Table 1.

Sampling Protocol

Samples of epilimnetic water were taken by hand approximately 10 cm below the surface of Lake 239 with a 2-L Nalgene bottle that had been rinsed twice with sample water. The samples were processed within 4 h after collection. High-DOC water, used for the DOC concentrate, was collected in a pre-rinsed carboy from the outflow of a sphagnum bog flowing into Lake 239. The bog water, and especially the concentrate, was brown in colour, indicating the presence of humic substances.

Sedimenting particles were collected from the hypolimnion of Lake 302N using cylindrical (60 cm × 10 cm in diameter) sediment traps. Particulate matter was removed from the lake every 7–10 d, refrigerated, and used within 1–2 d to equalize decomposition (respiration) rates in some experiments.

TABLE 2. Chemical composition of Lake 239 water amended with cation-exchanged DOC concentrate. Values are approximate composition of "highest [DOC]" for experiment 2.

Chemical	Concentration (μmol·L^{-1})
DOC	3100
Al	2.5
Cu	0.02
Fe	1.3
Mn	0.01
Mg	0.14
Na	190
K	0.15
Cd	0.003
Ca	0.20

Dissolved Organic Carbon (DOC)

The DOC concentrate was obtained by rotary-evaporating the sphagnum bog outflow water at 60°C to concentrate DOC. The concentrate was then passed through a cation-exchange column to replace cations with H^+.

DOC was defined as all forms of organic carbon passing through a glass fibre filter (Whatman GF/C, Fisher, 0.7–1.0 μm pore size) and was not further characterized. The concentrate was dark brown and likely contained high concentrations of humic substances. DOC was by far the dominant solute in the bog water used (Table 2). DOC concentrations were measured in GF/C-filtered sample water, after high-temperature acid persulphate digestion, by infrared detection of CO_2 on a model 700 Carbon Analyzer (OI Corp., Houston TX).

The DOC concentrate was not heat-sterilized because this would have chemically altered the DOC and destroyed the natural flora. The intent of our experiments was to simulate the natural environment, where high-DOC runoff mixes with lower DOC lake water.

Experimental Design

DOC concentrations in these experiments were obtained either by using lake and bog water directly or by diluting the

TABLE 3. Description of Experimental design.

Experiment	Objective	Design
1	Influence of DOC on methylation and demethylation. Natural pH, and at constant respiration rate	3 DOC concentrations used. DOC from bog outflow water diluted with lake water. Respiration held constant with 6 mg sediment trap (ST) material. Treatments: DOC 500, 1150, 2600 μM; unamended pH 5.8, 5.2, 5.0
2	Influence of DOC on methylation and demethylation. pH uniform, and respiration not constant	4 DOC concentrations used. DOC concentrate diluted with lake water; no ST material added. Treatments: DOC 560, 760, 1600, 3100 μM; pH = 6.0 ± 0.2 in each
3	Influence of DOC and pH on methylation, demethylation, and respiration	2 DOC concentrations and 2 pH levels used. DOC concentrate diluted with lake water; no ST material added. Treatments: DOC 500, 2600 μM; pH adjusted to 5.0 and 7.0 for each [DOC] (4 treatments)

DOC concentrate with lake water. Experimental designs are summarized in Table 3.

Decontaminated glass bottles containing 100-mL samples were incubated for 24 h for methylation and demethylation experiments. For respiration measurements, 50-mL glass syringes containing samples from the same batch solution as for the methylation and demethylation bottles were incubated under identical conditions.

For experiment 1, a slurry of sediment trap material equalized respiration among the three DOC treatments. the samples were composed of 1:1 Lake 239 water and sediment trap material (500 μm DOC), 1:1:2 Lake 239 water, bog water, and sediment trap material (1150 μM DOC), and 1:1 bog water and sediment trap material (2600 μM DOC). The incubation temperature was the same as the lake water temperature at 18°C; pH was not adjusted (Table 3).

For experiment 2, the Lake 239 water was amended with aliquots of DOC concentrate to achieve a natural range of DOC concentrations (Table 3). The lowest DOC concentration of 560 μM was unamended lake water, and the highest of 3100 μM was 1:9 DOC concentrate and lake water. The incubation temperature was 20°C; pH was adjusted to 6.0 with dilute NaOH or HCl.

In experiment 3, four treatments consisted of low and high DOC concentrations and pH levels of 5.0 and 7.0 (Table 3). As in experiment 2, the low DOC concentration was the natural Lake 239 DOC concentration (500 μM), while the higher DOC concentration (2600 μM) was a mixture of DOC concentrate and lake water. Each batch was halved, with the pH of one of each DOC concentration adjusted to 5.0 and the others to 7.0 with dilute NaOH or HCl. The incubation temperature matched the actual lake water temperature for the season at 25°C.

For each of the three sets of experiments, short-term methylation, demethylation, and respiration measurements were made as described below. Differences in sample water sources over time (temperature, bacterial numbers, chemical variables, etc.) disallow making any direct comparisons among rates found in the separate experiments. Only within-experiment comparisons can be made. Significant differences ($P \leq 0.05$) among treatments within an experiment were evaluated by analysis of variance (ANOVA).

Specific Rates of Mercury Methylation and Demethylation

Specific rates of mercury methylation were determined by the radiochemical method of Furutani and Rudd (1980), by adding 1.0 or 2.0 μg of Hg(II) (0.037 or 0.074 MBq as $^{203}HgCl_2$, New England Nuclear Corp.) to 100 mL of water. Briefly, the percent of added mercury that is converted to methyl mercury over a 24-h incubation period is quantified. All glassware was thoroughly cleaned and decontaminated by soaking overnight in a 2% Decon (BDH Laboratories) solution. The final rinse was the sample water that contained the natural microbial flora used in our experiments. The vessels used for sample incubation were 300-mL glass bottles with ground-glass stoppers. Two or three replicate samples plus one acid-killed control were incubated in the dark for 24 h under the conditions previously described and then were killed with 1.0 mL of 4 N HCl. $CH_3{}^{203}Hg^+$ extraction efficiency is essentially 100% even in very highly organic matrices such as sediments and fish tissue (Furutani and Rudd 1980), so it was assumed not to vary over the range of DOC concentrations used here.

Specific rates of demethylation were measured by addition of 0.2 μg Hg(II) (7.4×10^{-5} MBq as ^{14}C-labelled methyl mercuric iodide, Amersham Laboratories) to 100 mL of sample and recovery of volatile ^{14}C ($^{14}CO_2$ and $^{14}CH_4$, M. R. Winfrey, University of Wisconsin, La Crosse, WI, pers. comm.). The original procedure was fully described by Ramlal et al. (1986), who determined that the method recovers 99.7% of the volatilized ^{14}C. The sample replicates, experimental conditions, and time of incubation were the same as for methylation, except that the 4 N HCl added to terminate was added by injection through the stoppers so as to prevent escape of ^{14}C gases.

The term "specific rates," when referring to methylation and demethylation, means the percent of the isotope added that was methylated or demethylated. The $^{14}CH_4Hg^+$ is more available to the microbes than the $^{203}Hg^{2+}$, so a greater percentage of the labelled substrate is typically demethylated than methylated (Ramlal et al. 1986). The rate of methylation/rate of demethylation (M/D) ratios were <1 and are meaningful in a comparative rather than an absolute sense. Because ambient mercury or methyl mercury concentrations were overwhelmed by the radioisotope and carrier, our methods provided rates specific to the amount added rather than an in situ rate. Within each methylation and demethylation experiment, the amount of mercury added was kept constant. Thus, the effect of other variables on the specific rates of methylation and demethylation (and their ratio) could be studied.

Respiration

Respiration rates were measured by incubating duplicate 50-mL water samples in glass syringes from the same treatment batch under the same incubation conditions as for methylation experiments. In experiments that included sediment trap material (experiment 1), samples were incubated in the dark for 18 h at 18°C, while those without particulates were incubated 24 h at either 20°C (experiment 2) or 25°C (experiment 3). Initial

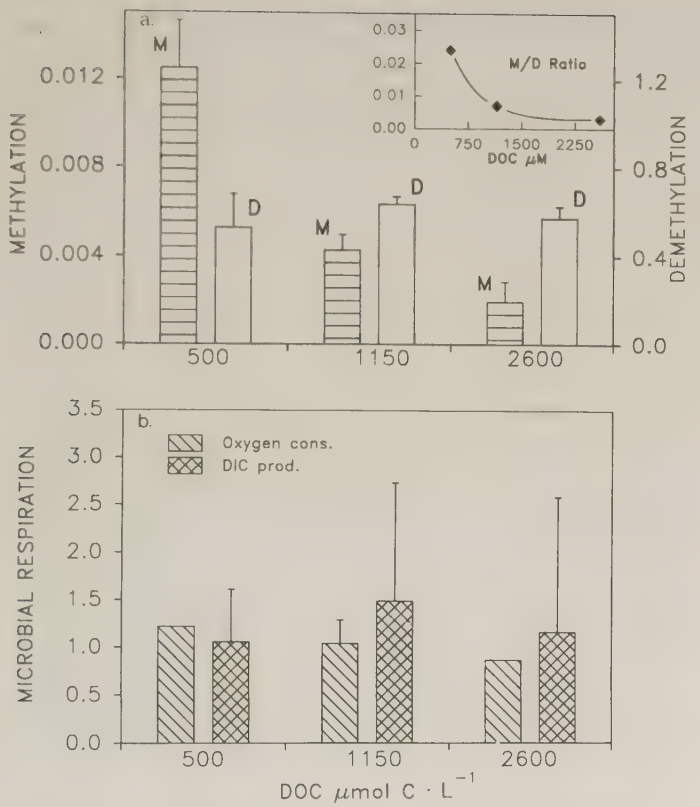

FIG. 1. (a) Rates of methylation (*M*, left axis) and demethylation (*D*, right axis) with DOC concentration. Units are $\%\cdot L^{-1}\cdot h^{-1}$. Standard deviation shown. Inset: ratio of rates, *M/D*. (b) Rates of microbial respiration with DOC concentration. Units are $\mu mol\cdot L^{-1}\cdot h^{-1}$. Additions of sediment trap material to all samples held respiration approximately constant for all DOC treatments.

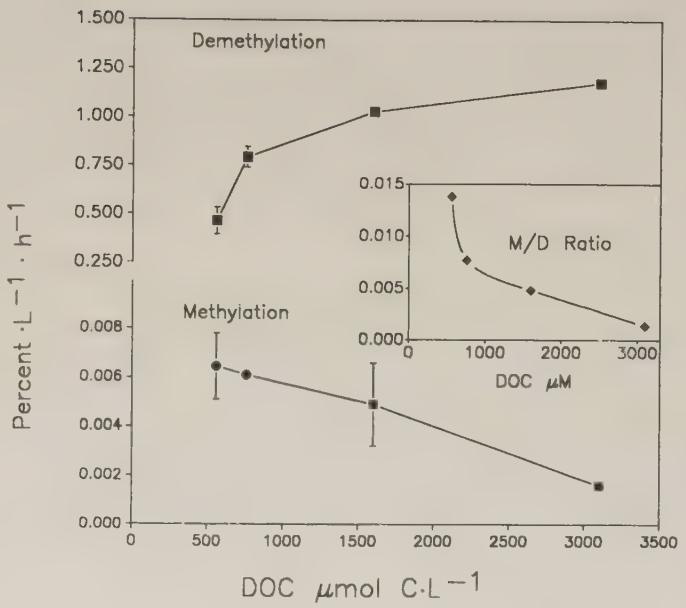

FIG. 2. Rates of methylation and demethylation with increasing DOC concentration. Respiration increased only between the lowest DOC concentration and each of the others. Standard deviation shown. Inset: ratio of rates, *M/D*.

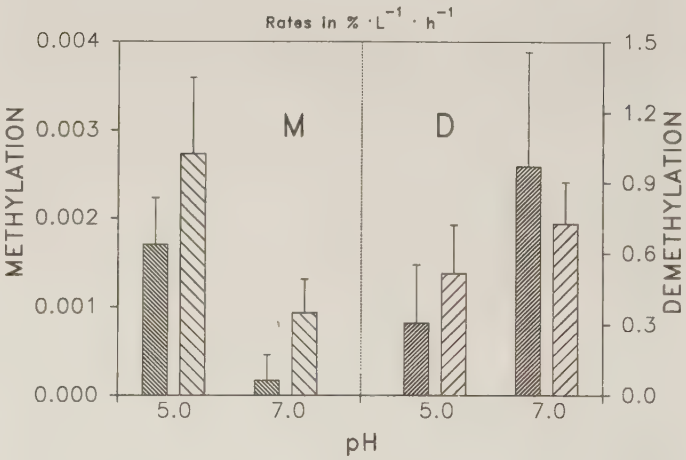

FIG. 3. Rates of methylation and demethylation at 2600 μM (light hatched bars) and 500 μM (heavy hatched bars) DOC. Rates are for pH 5.0 and 7.0. Standard deviation shown.

and final dissolved inorganic carbon (DIC) concentrations were measured with an infrared spectrophotometer on initial and final 0.5-mL aliquots (Stainton et al. 1977) that had been removed from the 50-mL sample syringe. Oxygen consumption rates were quantified using a microscale Winkler technique (American Public Health Association 1972) on initial and final 10-mL aliquots of the sample and using phenylarsine oxide (Hach Chemical Co.) as the titrant in place of sodium thiosulphate.

Results

In experiment 1, there were two- to threefold decreases in rates of methylation at the higher DOC concentrations (Fig. 1a). There were no detectable differences among rates of demethylation for each treatment (Fig. 1a). Respiration rates showed no trend with DOC concentration because of the added sediment trap material (Fig. 1b).

In the second set of experiments, pH was kept constant, no sediment trap material was added, and DOC was varied. DIC production increased from approximately 0.08 $\mu mol\cdot L^{-1}\cdot h^{-1}$ in 560 μM DOC to 0.8 $\mu mol\cdot L^{-1}\cdot h^{-1}$ at all of the higher concentrations. Oxygen consumption increased from undetectable at 560 μM to approximately 1.2 $\mu mol\cdot L^{-1}\cdot h^{-1}$ at all higher DOC levels. The opposite effect was seen for specific methylation rates. They decreased consistently with increasing DOC concentration (Fig. 2). This was the same methylation trend with DOC concentration as was found in experiment 1, where respiration was uniform due to sediment trap additions (Fig. 1).

The methylation rate was significantly lower at the highest DOC concentration (3100 μM) than at all of the other DOC concentrations. Demethylation rates increased significantly with each increase in DOC concentration. The largest increase in demethylation was between 560 and 760 μM (similar to respiration).

In both of the above experiments the *M/D* ratio, which is an indication of the relative potential for net CH_3Hg^+ production, was clearly highest at the lowest DOC concentration and decreased with increasing DOC. This occurred whether or not sediment trap material was present (Fig. 1a and 2 (inset)).

When both pH and DOC were adjusted (experiment 3), methylation and demethylation were influenced more by pH (Fig. 3 and 4) whereas respiration was influenced more by DOC (Fig. 5). Methylation rates were higher at pH 5.0 than at 7.0, but were suppressed by high DOC at both pH levels. Demethylation rates were higher at pH 7.0 than at pH 5.0 but did not

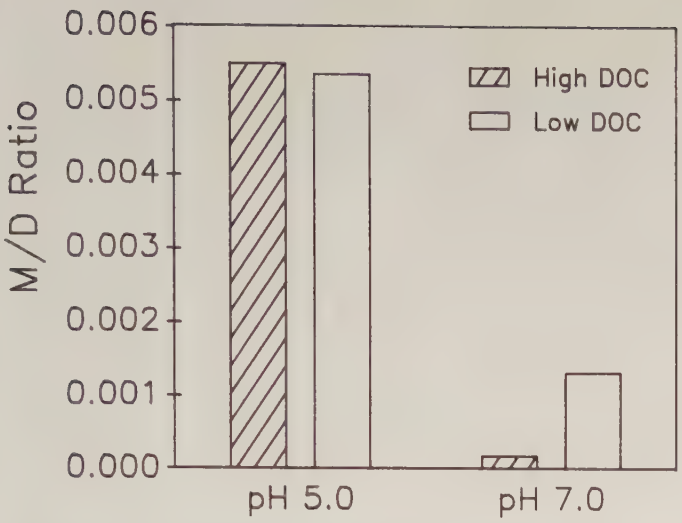

FIG. 4. Ratio of methylation to demethylation (*M/D*) at 2600 μM (hatched bars) and 500 μM (open bars) DOC, pH 5.0 and 7.0

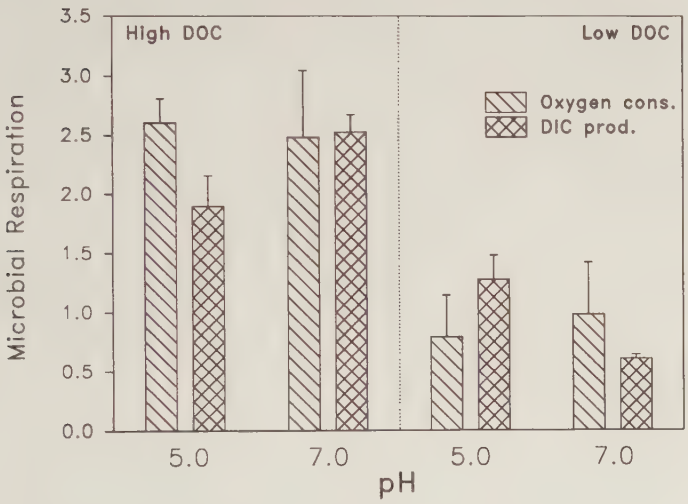

FIG. 5. Rates of microbial respiration (oxygen consumption and D/C production) at 2600 and 500 μM DOC in water at pH 5.0 and 7.0. Standard deviation shown.

follow a consistent pattern with respect to DOC concentrations (Fig. 3). The *M/D* ratios indicated a higher net potential for CH_3Hg^+ production at pH 5.0 than at pH 7.0 regardless of the DOC concentration (Fig. 4).

Respiration (oxygen consumption, DIC production) was significantly higher in the high-DOC (2600 μM) treatment than in the low-DOC (500 μM) treatments regardless of pH (Fig. 5). There was no significant difference in short-term respiration between pH 5.0 and pH 7.0 for any one DOC concentration.

Discussion

Among the three factors that we studied, the order of importance in affecting rates of net methyl mercury production was pH > DOC concentration > microbial respiration (Fig. 1–5). The fact that pH had the greatest effect is consistent with a number of fish surveys in which pH was the chemical variable that correlated most significantly with fish mercury (Wren and MacCrimmon 1983; Hakanson et al. 1988; Cope et al 1990).

The mechanism of the pH effect on methylation is unknown. However, the results from our DOC experiments suggest that a DOC-mediated mechanism is possible. Reduction in pH changes the character of DOC by increasing protonation of anionic moieties and thus desorbing metals (Davis et al. 1985). Therefore, the increased methylation at low pH in our experiments with lake water and by Xun et al. (1987) in water and at the sediment–water interface might be explained by reduced binding of inorganic mercury to DOC, making the mercury more available for methylation. Other possible explanations are that low pH favours activity of certain microbial species, or the operation of biochemical pathways that are effective at methylating mercury.

Additions of DOC to our samples caused enhanced microbial respiration (Fig. 5), as has been observed by others (Sederholm et al. 1973; Tranvik 1988; Tranvik and Hofle 1987). Demethylation appeared to follow microbial respiration; it was constant when respiration was constant (experiment 1) and increased when respiration increased (experiment 2). However, any stimulatory effect of increased respiration on demethylation rates or *M/D* was overwhelmed by the large inhibitory effect of DOC on methylation rates and *M/D* (Fig. 2). The inhibitory effect of DOC on methylation and *M/D* (but not on demethylation) was independent of microbial respiration in the experiment where the microbial respiration was held constant by additions of sediment trap material to overwhelm changes in substrate availability at the different DOC concentrations (Fig. 1).

The mechanism by which DOC inhibits biotic methylation of mercury is unknown. The most likely explanation is that the availability of Hg^{2+} was reduced by binding of the free mercury ions to DOC (Kerndorff and Schnitzer 1980; Lodenius et al. 1987; Jackson 1989). Another possibility is that the added DOC inhibited the methylating bacteria even though overall microbial respiration rates were elevated.

Other researchers (Nagase et al. 1982; Lee et al. 1985) have found that DOC stimulates abiotic methylation at very high DOC concentrations ($>14 \times 10^3$ μM). While this may result in substantial methyl mercury production in very high-DOC soil porewater, in our experiments, the fact that methylation rates were inversely proportional to DOC concentration suggests that biotic methylation was predominant in our aquatic samples. This observation is in agreement with the conclusions of Berman and Bartha (1986) and Jackson (1989) that the significance of abiotic methylation in lake sediments is minor in comparison with microbial methylation.

The results of the short-term experiments presented in this paper cannot necessarily be extrapolated to predict long-term effects in lakes with differing pH levels and DOC concentrations. However, these results do suggest several testable hypotheses that might explain high mercury concentrations in fish taken from different types of lakes. On the basis of our experiments, we hypothesize that high mercury concentrations in fish from clear-water, acidified lakes are caused by increased in-lake methylation in the water column and at the sediment–water interface because of decreased binding of Hg^{2+} by DOC at low pH. We hypothesize that in brown-water, low-pH lakes, high fish mercury concentrations may originate from methyl mercury transported from the drainage basin as DOC complexes (as proposed by Lee and Hultberg 1990), as well as from in-lake methylation at low pH and high DOC (Figs. 3 and 4). We further hypothesize that the negative correlation between fish mercury and DOC in seepage lakes found by Grieb et al. (1990) could be explained if most of the methyl mercury in

seepage lakes is produced in situ rather than in the watershed and if the in-lake methylation is reduced, as we have observed, by DOC concentrations.

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Acoustic Observations of Patterns of Aggregation in Haddock (*Melanogrammus aeglefinus*) and Their Significance to Production and Catch[1]

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Boudreau, P. R. 1992. Acoustic observations of patterns of aggregation in haddock (*Melanogrammus aeglefinus*) and their significance to production and catch. Can. J. Fish. Aquat. Sci. 49: 23–31.

A dual-beam acoustic system was used to collect detailed information on density and body size over a 24-h period in an area of the Scotian Shelf occupied by a population of large spawning haddock (*Melanogrammus aeglefinus*). The acoustic sampling showed a persistent gradient of decreasing density with distance from bottom with 50% of the fish <2.5 m off bottom. Horizontally (scale of hundreds of metres), there was a high degree of local aggregation which changed dramatically over 24 h. Highest aggregation was in daytime, with fish more dispersed throughout the water column at night. The differences in the degree of aggregation with time of day appear to be sufficient to explain diel changes in net catch rates. On larger geographic scales (tens of kilometres), fish density was relatively uniform. Acoustics provide a method for studying the relative importance of aggregation on these various spatial scales to estimates of abundance. Haddock biomass density was also related to organism body size, as has been observed for other species in both aquatic and terrestrial situations, suggesting that haddock population density is determined by trophic interactions similar to those that underlie production of other populations of organisms.

Un système acoustique à double faisceau a été utilisé pour obtenir des données détaillées, au cours d'une période de 24 h, sur la densité et la taille de gros aiglefins (*Melanogrammus aeglefinus*) d'une population en frai dans une zone de la plate-forme néo-écossaise. L'échantillonnage acoustique a montré l'existence d'un gradient persistant de baisse de densité en fonction de la distance au fond, 50 % des poissons se trouvant à moins de 2,5 m du fond. Il a été noté, dans le plan horizontal et à une échelle de centaines de mètres, un degré élevé de concentration qui s'est modifié de façon très importante au cours des 24 h. La concentration la plus importante a été notée pendant le jour; les poissons étaient plus dispersés dans la colonne d'eau pendant la nuit. Les écarts de concentration en fonction du moment de la journée semblent suffisants pour expliquer les variations nycthémérales des taux de prises au filet. À une échelle géographique plus importante, de l'ordre de dizaines de kilomètres, la densité des poissons apparaissait relativement uniforme. Les relevés acoustiques permettent d'étudier l'importance relative des concentrations à ces diverses échelles spatiales et ainsi d'estimer l'abondance. La densité de la biomasse des aiglefins présentait aussi un lien avec la taille des poissons, comme cela a été observé pour d'autres espèces tant en milieux aquatiques que terrestres, ce qui porte à croire que la densité des populations d'aiglefin est déterminée par des interactions trophiques semblables à celles agissant sur la production d'autres populations d'organismes.

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The distribution of organisms in space, through its impact on the costs and benefits of predation, is an important determinant of population success as reflected in various measures of production (Ivlev 1960, 1961, 1966). The importance of distribution is underlined by the pervasive patchiness that characterizes populations of organisms ranging in size from microflagellates (Goldman 1984) through zooplankton (McLaren 1963; Enright 1977; Enright and Honegger 1977) to whales (Brodie 1975; Gaskin 1978). In all such studies it is evident that interpretation of observed changes in local density, in terms of understanding organism energy requirements, dictates extensive vertical and horizontal sampling. The required degree of detail is most readily achieved through new data collection and analytic technologies.

This is particularly evident in recent studies of marine fish production where it has been found necessary to distinguish the effects of catchability and aggregation from those of abundance changes in explaining fluctuations in commercial catches (Winters and Wheeler 1985; Crecco and Overholtz 1990). Even where information essential to measuring abundance changes is available, such as the positions of ecosystem boundaries and average fish densities, sampling with nets, such as otter trawls, still operates on horizontal and vertical scales that are both too coarse and too selective to explain short-term differences in catch or to allow study of the factors determining biological production.

In this paper, data from a 24-h acoustic survey are analyzed to provide detailed information on individual fish size and local density along a set of moderately long acoustic transects within an area of relatively high abundance. The acoustic data are compared with results from net samples taken at the same time and with survey results over larger geographic areas. The results are used to interpret well-known patterns of change in both

[1]S.W. Nova Scotia Fisheries Ecology Project Contribution No. 21.

commercial and survey catch rates, in terms of aggregation of the population. Indices of aggregation by body size calculated from the acoustic data also appear useful in explaining their relation to the factors controlling fish population production.

Methods

In March 1985, a series of acoustic transects and net hauls was made over a 2-wk period using a dual-beam acoustic system and a Yankee 36 bottom otter trawl from the R/V *E. E. Prince*. A concentration of large demersal haddock (*Melanogrammus aeglefinus*) was found east of Browns Bank on the western Scotian Shelf where in the spring they customarily form spawning concentrations. Depth was about 100 m and bottom topography was flat and amenable to trawl sampling. Net hauls made on the apparently highest concentrations caught only haddock with a modal length of 50 cm. For one day–night cycle, eight 20-km acoustic transects were made in a perpendicular pattern centred at about latitude 42°45′00″N and longitude 65°00′00″W. Ship speed was held constant at about 10 knots. At intervals during daylight hours, three additional short acoustic transects were completed simultaneously with net trawling on the area of highest fish concentrations. For these transects, ship speed (3.5 knots) and distance (3.5 km) followed standard scientific net survey procedures. Data from the nets verified that species composition, overall population density, and size composition remained stable over the period.

The ECOLOG I hydroacoustic system was deployed to collect data on density and body size for the bottom 30 m of the water column. This system, described by Dickie et al. (1983, 1984) and Dickie and Boudreau (1987), employs counting algorithms for calculating the density of separated fish targets (Ehrenberg 1979) such as are characteristically observed with large demersal fish. The dual-beam configuration (Traynor and Ehrenberg 1979) allows calculation of acoustic size of the target independently of the effects of beam pattern.

The dual-beam ECOLOG I system uses two 50-kHz transducers, the narrow and wide beams being aligned on the same acoustic axis. They are deployed in an outboard towed body oriented so that the pulses of acoustic energy propagate vertically through the water column towards bottom. Pulses of 0.3 m length are transmitted on the narrow-beam transducer for 400 μs twice a second. Sampling for reflected acoustic energy is carried out on both transducers at a rate of 10 kHz and all signals above a preset detection threshold are stored digitally on 9-track magnetic tape. Limited data processing was carried out in real time to control sampling. Detailed analysis was undertaken at the completion of the cruise.

Processing of the acoustic data was designed to differentiate and classify signals into discrete targets using information on range and beam width (Dickie et al. 1983). Relative horizontal position of the single fish targets along the transect was determined from ship speed, pulse transmission rate, and pulse number. Vertical position of targets in the water column was calculated from the difference in ranges from the transducer to the target and to bottom.

Acoustic size determination for fish targets in terms of target strength (TS) depends on corrections of the received signal for several known sources of variation. Those related to the electronic system, the acoustic system, and the physical environment are well known and are relatively easily made (MacLennan 1990). The necessary system calibration parameters for this cruise were determined using facilities made available by the Defense Research Establishment - Atlantic. Two additional major sources of variation that are more difficult to account for are functions of the fish target itself: position in the beam and fish aspect tilt angle (Dickie and Boudreau 1987; Thiebaux et al. 1991). Although these target effects can be measured independently in the laboratory, in the field, where the transducer is being towed relatively rapidly over nearly stationary fish targets, these effects become combined into what may be termed perspective effects (Dickie and Boudreau 1987).

The sizing algorithm developed for use in this study makes use of the fact that during the survey, most single fish targets were seen repeatedly from a series of perspectives. Averaging of these replicate measures for single fish targets was used to reduce the effects of noise so as to obtain an improved estimate of TS.

Estimates of average body size in acoustic and net samples are calculated from the frequency distribution of numbers density by logarithmic body length groups. The logarithmic transformation of body length was found to normalize the size frequency distribution of fish, which was otherwise highly skewed towards the small sizes. The acoustic and net estimates of average log size were found to be highly correlated (r = 0.72) (Dickie and Boudreau 1985; Boudreau 1989). For this cruise, a field calibration of the length estimation algorithm was made by comparing the acoustic average with that from net catches made over the course of the 2-wk cruise.

To calculate numerical fish densities from the acoustics it is necessary to determine the volume of water in which a given target can be detected. The linear distances sampled during a transect were readily calculated from Loran C positions at the beginning and end of transects. However, the width of the transect is a function of both target size and range from the transducer (Kieser and Mulligan 1984). In this paper, transect width is estimated from information on the transducer beam pattern, target range, detection threshold, and the apparent acoustic size. Five-centimetre length groupings are used here for calculating numerical density over specified depth zones.

The vertical position of a taget in the water column is readily determined from the range. The vertical depth zone over which average densities were calculated depends on the comparisons being made. To study overall distribution by depth, the acoustic data were divided into two principle depth zones: the bottom 2.7 m water layer corresponding to the layer sampled by the bottom trawl, and the water column from 2.7 up to 30 m, which provided information on haddock not sampled by the net. The volume sampled by the Yankee 36 trawl was calculated from the dimensions of the net (2.7-m headline height and 12-m wingspread) given by Kohler and Smith (1983). Capture efficiency was assumed constant for all fish sizes and densities.

Acoustic data on fish size and numerical density can be compiled for various horizontal distances as well as depth zones. To characterize the horizontal distribution of the haddock, density was calculated for the bottom 30 m of the water column because this depth zone contained 99% of the observed fish. The best unit length of horizontal transect segment varied with the comparisons being made. As is pointed out in the results, to study small-scale horizontal aggregation patterns, 100-m horizontal segments of the acoustic transects appeared most appropriate. For comparison with net sampling, an intermediate sampling segment of 3.5 km was used. Results for this segment length could be related to the effects of aggregation on both standard scientific net sampling and on commercial catches. Average population density and average body size calculated

for the entire 20-km length of transects appeared to give estimates that best reflect average abundance in the whole geographic area.

Lloyds index of crowding, p, was used as a statistic to quantify the degree of fish aggregation along the transects. For this index, the mean number of fish, $\bar{x}$, and variance, σ^2, for the segments of a transect or net hauls within a stratum were used to calculate p:

$$(1) \quad p = 1 + (\sigma^2 \bar{x}^{-1} - 1)\, \bar{x}^{-1}.$$

For a normal distribution, where the variance equals the mean, $p = 1$. This statistic was chosen because of its independence of sampling scale (McGurk 1986). Two transect segment lengths, 100 m and 3.5 km, were used to compare the degree of aggregation on two different spatial scales. The statistics calculated from the acoustic data using the longer 3.5-km segments were also compared with those calculated for haddock catches from three net surveys designed to measure abundance: a February 1985 groundfish cruise and a July 1985 groundfish cruise, both on the Scotian Shelf in the vicinity of the acoustic survey, and a Georges Bank survey reported by Taylor (1953). Data for the Scotian Shelf cruises were taken from DFO, Marine Fish Division STRAP reports (See Halliday and Koeller (1981) for details regarding sampling methods and strata description).

Results and Discussion

Small-Scale Patterns of Aggregation

Measures of aggregation depend on the transect segment length chosen as a sampling unit. Preliminary scanning of the acoustic data from successive pings, each of which covered a diameter on bottom of about 10 m along the 20-km transects, showed that, during daytime, fish aggregations were characteristically less than 100 m in length. That is, a transect segment of 100 m in length containing an aggregation was usually preceded and followed by segments with low or zero fish densities. Comparison of the mean and variance of fish densities for a variety of daytime segment lengths, from 1 to 1000 m, showed that grouping the data by 100-m segments tended to maximize the variance/mean ratio. As a result the 100-m segment length was adopted as the basic unit in describing small-scale fish aggregation.

The frequency distributions of local fish density in 100-m segments, calculated for the bottom 30 m of the water column, fell into three time of day classes. Transects carried out at night, between 21:00 and 06:00, had the highest overall mean density with a modal class of 51–75 $N/10^5$ m^3, a maximum density of only 225 $N/10^5$ m^3, and very few (0.7%) segments where no fish were encountered (Table 1). By contrast, 50.0% of the daytime segments contained no fish, but where fish were encountered, densities were high, sometimes more than 500 $N/10^5$ m^3. At dusk, a relatively high proportion of segments had a low or zero density, but only 0.6% of dusk segments had densities higher than 225 $N/10^5$ m^3. This change in degree of aggregation was also reflected in the decrease in the variance/mean ratio from day to night (Table 1): however, even for nighttime, the variance remained more than 20 times the value of the mean, indicating persistent aggregation throughout the 24-h period.

During the day, the fish were found in concentrations off bottom (Fig. 1A). These appeared to be associated with high densities near bottom suggesting a significant vertical component in the patterns of aggregation in the daytime. At nighttime the pelagic concentrations had disappeared leaving a gradient of increasing fish density with proximity to bottom (Fig. 1B). When the data for both day and night were compiled into the two depth zones, one close to bottom which was sampled by the nets and one above it, densities near bottom were significantly higher (Table 2). For all times of day, approximately 50% of the fish seen in the water column were within 2.7 m of bottom (Table 2). With this vertical structuring, indices of abundance based on sampling a single depth slice need to be carefully scrutinized.

Changes in density with distance off bottom appeared to be accompanied by changes in the average fish size (Table 2). Acoustically estimated sizes of fish up in the water column were on average estimated to be more than 10 cm smaller than those near bottom. Nighttime estimates of average fish size were 6 and 9 cm smaller for the bottom and off-bottom layers, respectively, than the daytime estimates.

Some of the apparent vertical differences in average size could be artifacts of the acoustic methodology, through the effect of fish tilt angle on TS. Tilt distributions at sea are known to vary (Buerkle 1983), and Foote (1980, 1985) has shown how the distribution of tilt angles affects acoustic size. The algorithms used here for calculating perspective-corrected average acoustic size markedly reduce the variance of TS, but do not account for the effects of a higher tilt angle variance on average size. Thus, differences in size between the two depth layers may result from greater variation in the tilt angles for fish in the pelagic mode compared with those near bottom and oriented in relation to it. The slight decrease in average size from day to dusk and night in the bottom depth zone may similarly result from a decreased ability of fish to maintain uniformity in horizontal orientation under reduced light levels.

Without independent field data or more extensive data analysis (Thiebaux et al. 1991), changes in acoustic size due to changes in tilt angle cannot be certainly distinguished from real changes in biological size. However, in keeping with observations of cod spawning behaviour, the concomitant decrease in average acoustic size for both depth zones from day to night supports the hypothesis that the observations reflected real changes in size. Brawn (1961) described the effect of size dominance of large spawning cod (*Gadus callarias*) on vertical distribution. The decrease in acoustic size in both water layers may similarly reflect nighttime vertical migration of the larger of the spawning haddock from the upper water column towards bottom, thereby reducing the apparent average size in both depth zones.

Effects of Aggregation on the Estimation of Abundance

Estimation of abundance requires that information on local density be averaged appropriately over the area or volume occupied by the population. In the acoustic survey the average densities for various transects show significant differences in relation to time of day. Of the three time of day sampling periods, highest total numbers and significantly higher densities were observed in the nighttime samples near bottom (Table 2). Differences between night and daytime density estimates were also statistically significant for the upper depth zone.

It seems clear that these apparent differences in abundance with time of day resulted from the patchiness of the fish distributions. The population in daytime was highly aggregated into small water volumes, with a consequent low probability

TABLE 1. Percent frequency of fish densities for 100-m segments derived from acoustics. Transects are classed by time of day. *N* is the number of fish observed in each density class. Average acoustic length is given for each density class.

Density class ($N/10^5$ m^3)	Day			Dusk			Night		
	Freq. (%)	*N*	Length (cm)	Freq. (%)	*N*	Length (cm)	Freq. (%)	*N*	Length (cm)
0	50.0	—	—	37.2	—	—	0.7	—	—
1–25	28.7	137	43	36.0	165	39	7.0	34	34
26–50	9.4	132	40	18.9	255	33	23.3	327	33
51–75	4.1	95	42	2.7	60	31	24.9	580	31
76–100	1.9	61	37	1.5	47	27	19.9	647	30
101–125	1.0	42	39	1.5	60	30	12.9	537	27
126–150	0.8	41	35	0.6	28	28	6.1	311	26
151–175	0.6	37	35	0.6	35	21	3.2	192	25
176–200	1.0	69	35	0.3	20	22	1.1	75	23
201–225	0.5	39	42	0.0	—	—	0.9	70	25
226–250	0.6	52	38	0.0	—	—	0.0	—	—
251–275	0.4	39	43	0.3	27	21	0.0	—	—
276–300	0.0	—	—	0.0	—	—	0.0	—	—
301–325	0.1	11	29	0.0	—	—	0.0	—	—
401–425	0.1	15	32	0.0	—	—	0.0	—	—
451–475	0.1	17	39	0.3	48	26	0.0	—	—
476–500	0.3	54	49	0.0	—	—	0.0	—	—
551–575	0.3	62	31	0.0	—	—	0.0	—	—
626–650	0.1	24	54	0.0	—	—	0.0	—	—
Mean density	25 ± 64			21 ± 40			75 ± 41		
Variance/mean	163			76			22		

of encountering population components in a given sample. For example, during the daytime, the numbers of fish observed in aggregations with densities of >225 $N/10^5$ m^3 made up 30% of the total, but such densities occurred in only 2.0% of the segments sampled (Table 1). That is, 33% of the observed fish were found in patches which occupied only 2.5% of the sampled area (Table 1). By contrast it was apparent from the much lower patchiness at night that means and variances for the nighttime sampling provided more stable and apparently more realistic estimates of population abundance.

Aggregation and Sampling with Nets

This high degree of aggregation also helps to interpret the net sampling results in which net estimates of density were five times higher than the acoustic estimates (Table 2). The three net samples were all completed in daytime on high concentrations identified using the echo sounder. Although the acoustic sampling on these transects also encountered the highest fish densities observed for the 2-wk cruise, and acoustic average densities on them were high compared with the densities calculated for the longer 20-km acoustic transects, the density estimates were significantly lower than the net estimates.

There are three possible explanations for the difference in density estimates by the two sampling systems. The first is that, at such high densities the acoustic system was "saturated." However, saturation, where two or more targets appear as one, would also result in significantly higher acoustic size estimates. Since the average size of fish was the same in both data sets, saturation does not appear to be the reason for the relatively lower acoustic estimates. A more likely explanation involves the estimate of the water volume sampled by the net. A constant width of net opening was assumed based on the physical dimensions, but it is known that the warps and doors of the trawl and the sediment cloud associated with it increase the sampling volume by herding fish (Ona 1990). This probable bias in volume sampled is difficult to test without direct observations of net fishing. A third possibility involves the increased probability of encountering fish aggregations in a trawl which samples a path greater than 12 m wide, compared with an acoustic track which reaches a maximum of 7 m near bottom. The results from both net and acoustics have been scaled to the equivalent volumetric units, but where the degree of aggregation is high, a doubling or trebling of the actual path of the net may well have a significant effect on the average density encountered. With present information it is not possible to evaluate the relative importance of these potential effects.

Aggregation on Geographic Scales

The significantly lower small-scale aggregation at night apparent in the visual analysis (Fig. 1) is also evident in the measures of Lloyd's index (p). Using 100-m segments the average p calculated for the nighttime transects was significantly smaller than values for day or dusk (Table 3). However, using the longer transect units of 3.5-km segments, comparable with the distances sampled in standard scientific net sampling, did not show any aggregation, and for all times of day, p for these segment lengths was low relative to values calculated using 100 m. The p value for the 100-m daytime segments was more than seven times that for 3.5-km acoustic segments. This confirms that, although p is scale independent, the selection of the basic sampling unit determines the capacity to identify certain scales of aggregation (Pielou 1974). The longer segments which smooth over the basic natural variability in local density do not provide information on the processes related to small-scale aggregation but would appear to be better designed to reflect an average density related to the level of relative abundance.

This same effect is also evident from a comparison of acoustic results with standard groundfish surveys for stratum 477

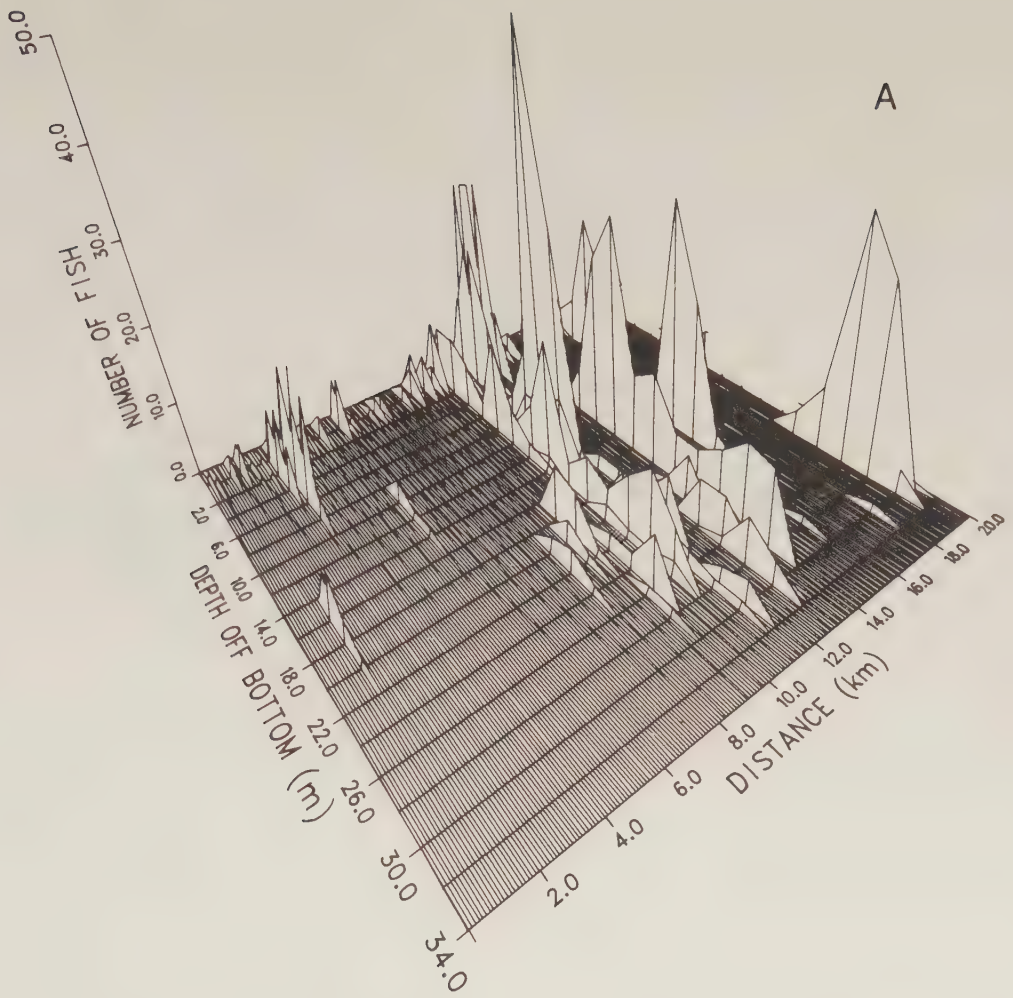

FIG. 1. Results from acoustic transects showing changes in aggregation. Plot shows fish density versus depth off bottom and distance along transect. Number of fish is calculated for 2-m depth slices and 100-m horizontal segments. (A) 20-km transect from daytime acoustic transect; (B) 20-km transect for nighttime acoustic transect. (*Fig. 1 concluded next page*)

within which the acoustic survey was completed (Table 3). Although the index for the net survey is based on only two trawl hauls in each cruise, estimates of p are within the range of values calculated from the acoustic survey using 3.5-km segments. Values of p did not vary much between the two cruises completed over 6 mo apart.

The patterns of aggregation within the 20 by 20 km area of high abundance can also be compared with distributional patterns prevailing over larger geographic areas. In Table 3, p is calculated from the groundfish trawl data over the whole Southwest Scotian Shelf, strata 471–485. The mean index calculated for the 11 and 13 strata sampled during the surveys, based on a total of 33 and 37 individual net samples, respectively, was significantly higher than that calculated within the smaller area. Similarly, both the mean and standard deviation of p calculated for Taylor's data for Georges Bank were higher than the estimates for the acoustic transects using the same sampling distance.

These results appear to reflect the greater heterogeneities expected in distribution over larger area and depth strata. Because the trawl surveys were not specifically directed towards haddock, they covered areas corresponding to habitats ranging from preferred haddock areas, in which there is a high degree of "crowding," to those not at all suitable for haddock. Haddock within the spawning region that was surveyed acoustically appeared to occupy the area relatively uniformly. It is clear that both the sampling method and the sampling scale of a survey will affect the efficacy of realizing survey design objectives.

Mobile commercial fishing fleets attempt to maximize catch rates, a problem which is the inverse of problems involved in representative scientific sampling. That is, by taking advantage of known patterns of aggregation into preferred areas or at particular times of day, commercial fleets can maintain catch rates at high levels relative to random fishing (Winters and Wheeler 1985). This is particularly important when stock abundance is decreasing and the distribution of the stock is contracted into areas smaller than the full potential environmental or geographic range. Crecco and Overholtz (1990) found a general insensitivity of catch statistics to changes in stock size because they were influenced more by the stable density than by the variable area occupied. It is on this same scale that Rose and Leggett (1988) were able to show the importance of environmental factors in determining fish distribution and interpreting the success of fixed fishing gear.

The acoustic measures of fish aggregation provide an explanation for changes in overall catch that are observed in fishing

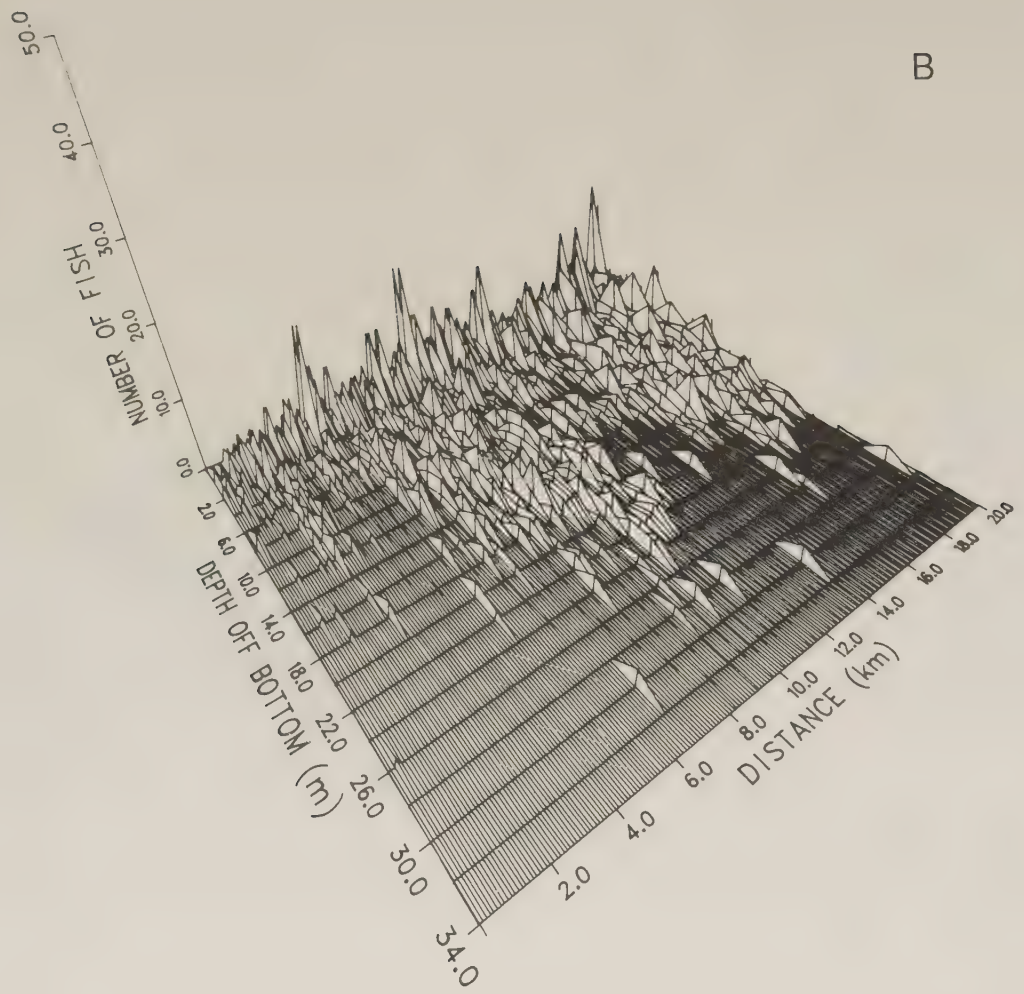

FIG. 1. (*Concluded*)

on smaller scales, within the major geographic areas of concentration. Daytime commercial trawls on haddock in the Browns Bank area are routinely completed within an hour of setting out. But the relatively high catch rates they experience then fall off at night, at which time individual sets are lengthened. In the extreme, when nighttime catch rates are very low, nighttime fishing activities may cease altogether with the object of cutting costs. Fishermen's experience that continued searching of extensive areas at night seldom discovers alternative aggregations and reappearance of high catch rates within a short time following sunrise is in accord with our deduction that the populations do not undertake diel geographic migrations but are merely undergoing changes in their degree of aggregation. This effect of aggregation on catch rate was predicted by the model of Paloheimo and Dickie (1964) which showed that aggregation and abundance have identical effects on catch rate. The acoustic sampling methodology provides the only direct measurement of the degree of aggregation on scales relevant to interpreting changing catches.

Density in Relation to Body Size

Population production and yield in nature is related to the density distribution of organisms and their body size as a result of the relation between their metabolic requirements and their ability to satisfy their food requirements from the prey populations (Dickie et al. 1987a). The acoustic information on density and body size shows that haddock distribute themselves in relation to body size in much the same way as do organisms in other situations. On the local scale, using data for 100-m segments (Table 1), average body size was strongly negatively correlated (up to $r = -0.90$) with local density between 1 and 225 $N/10^5$ m^3, for all time of day classifications. Above this density, particularly during the daytime, size varied greatly among the density classes, suggesting a strong tendency for the largest fish to school by size. For example, the daytime segment with the highest local density (625 $N/10^5$ m^3) also had the largest average fish length (54 cm). Overall, however, it appears that the smaller fish were more densely packed. This conclusion is in accord with the results of White et al. (1981) who observed haddock caught in individual trawl samples. They observed that large catches within an area of relatively stable abundance were dominated by smaller fish.

Considering that numerical organism density is an allometric function of body size, w (Damuth 1981), the acoustic data may be used to estimate coefficients a and b in the equation

$$(2) \quad \log N/\mathrm{m}^2 = a + b \log w.$$

Log numbers density for haddock of different sizes was highly correlated with log body size for all times of day (Table 4). The coefficients, a, are much lower for the fish data than those calculated for mammals by Damuth (1981), indicating a lower average density on this marine area for a given body size than

TABLE 2. Statistics for 20-km acoustic transects averaged within time of day classes for two depth zones. Results are also given for calibration transects where simultaneous acoustic and trawl sampling was carried out.

				Calibration results	
Classification	Day	Dusk	Night	Acoustic	Trawl
Time (h)	08:00–18:00	18:00–21:00	21:00–06:00	08:00–18:00	
Distance (km)	18.5	24.0	23.0	3.5	
Total number of fish	927	745	2773	175	1028
Number of transects	4	2	2	3	
Off bottom (>2.7 m)					
Overall density ($N/10^5$ m^3)	10 ± 4	9 ± 3	40 ± 5	20 ± 12	—
% number of fish	47	53	54	40	—
Average length (cm)	38	31	29	32	—
Near bottom (<2.7 m)					
Overall density ($N/10^5$ m^3)	104 ± 50	69 ± 18	241 ± 26	199 ± 12	1018 ± 45
% number of fish	54	47	46	60	—
Average length (cm)	48	41	42	51	50

TABLE 3. Statistics for the Lloyd's index of crowding calculated for the acoustic survey, two groundfish trawl surveys on the Scotian Shelf, and a trawl survey on Georges Bank. Lloyd's indices are calculated for the acoustic data using two segment sizes, 100 m and 35 km. Indices for trawl sampling are calculated within each stratum. The indices are averaged within the various classifications.

		Sampling segment				
		100 m		35 km		
Time of day/year	N	$\bar{p}$	SD	$\bar{p}$	SD	Source
Day	4	8.27	3.10	1.15	0.06	This paper
Dusk	2	4.82	1.96	1.52	0.03	This paper
Night	2	1.27	0.04	1.02	0.01	This paper
SW Scotian Shelf stratum 477						
Feb.	1	—	—	1.00	—	DFO STRAP reports
July	1	—	—	1.03	—	DFO STRAP reports
SW Scotian Shelf strata 471–485						
Feb.	11	—	—	2.00	0.84	DFO STRAP reports
July	13	—	—	1.74	0.55	DFO STRAP reports
Georges Bank						
1948–50	16	—	—	4.16	2.23	Taylor 1953

in the terrestrial ecosystems. Of importance here, the allometric exponent, b, ranges from -0.33 in the daytime to -0.76 for nighttime. Results for the nighttime transects are not significantly different from those of Damuth (1981) or Boudreau and Dickie (1989).

TABLE 4. Statistics for regression of numbers density with body size (eq. 2) for the acoustic data and two other studies.

		Coefficients			
	r	a	SE	b	SE
Time of day					
Day	−0.94	−2.21	0.11	−0.33	0.04
Dusk	−0.97	−1.44	0.15	−0.64	0.05
Night	−0.99	−0.51	0.07	−0.76	0.02
Damuth 1981	−0.86	4.23	—	−0.75	0.03
Boudreau and Dickie 1989				−0.75	

The spatial distribution of this marine fish population is evidently in keeping with the results of Boudreau and Dickie (1989) which is based on averages for a variety of fish species and of Damuth (1981) based on averages for several terrestrial grazing mammal species, implying that the same trophic mechanisms are operating here. However, the fish observations show differences in the estimated allometric exponent between day and night. While this difference may partly reflect limitations in the extent of the sampling effort, it is more likely that additional biological phenomena are involved. For example, the low daytime exponent occurs when the fish are most highly aggregated, suggesting that factors such as predator avoidance or, in the case of this haddock population, spawning activity may be operating on these sampling scales as short as a few hours and as small as kilometres, and so dominate the distribution pattern and obscure the relation with size at that time of day. This prominent daytime schooling behaviour appears to

be overlaid on the basic distribution related to trophic requirements, which appears at night as the predator avoidance or other intense schooling motivation is relaxed.

The distribution of organisms with body size is an important factor in fishery dynamics, and the demonstrated aggregation in haddock suggests that trophic interactions are involved in determining long-term fisheries yield (Boudreau and Dickie 1989). For example, the observed nighttime relationship between numerical density and body size ($N/m^2 \propto w^{-0.76}$) in haddock implies that the biomass density ($N/m^2 \times w$) is increasing as $w^{+0.25}$. As a consequence it appears that a catch of larger fish by a unit of fishing effort represents a higher fraction of the population biomass than does a similar catch of smaller sized fish (Dickie et al. 1987b). The ability of commercial fishing fleets to concentrate effort in areas of higher densities (Winters and Wheeler 1985), particularly during periods of low abundance, would thus place a selectively high fishing mortality on those larger individuals.

Conclusion

The acoustic data provide useful information on the scales of aggregation which affect net catch rates and suggest that the effects depend on the spatial dimensions of the net path relative to the area occupied by a school. For example, the abundance estimates made from daytime catch rates of randomly placed 3.5-km scientific sampling net hauls were exposed to biases as a result of strong daytime aggregation of fish into restricted areas. The result was a low probability that a single net sample would encounter sufficient elements of the population to be representative. Under such conditions, when abundance is low, selectivity by fish for good habitat would give rise to additional biasses. By contrast, it is apparent that acoustic information on distribution can be used to determine the appropriate horizontal sample sizes for estimating abundance by permitting calculations of the spatial sampling scales that are least influenced by aggregation (MacLennan and MacKenzie 1988). Additional information of vertical aggregation afforded by acoustic sampling needs also to be integrated into such estimates of fish abundance.

The acoustic results obtained here provide the possibility of estimating the three observable parameters necessary for calculating population production and yield: abundance, body size, and distribution (Boudreau et al. 1991; Crecco and Overholtz 1990). It is only through direct observations of these parameters that theoretical developments related to ecological production mechanisms (Boudreau et al. 1991) can be tested. If additional information continues to show the importance of organism body size and density distribution in determining production, it would appear that in acoustic sampling we have a valuable addition to traditional net sampling and stock assessment techniques.

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Mapping, Estimating Biomass, and Optimizing Sampling Programs for Spatially Autocorrelated Data: Case Study of the Northern Shrimp (*Pandalus borealis*)

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The methodology for mapping and for global and cutoff estimation of autocorrelated exploitable resources is presented, based on stationary geostatistical methods. Use and performance of these methods in marine ecology are illustrated with an application to northern shrimp (*Pandalus borealis*) abundance data, collected in 1989 at 137 stations in the western Gulf of St. Lawrence. Nonstationarity of the biomass data, a proportional increase of the local variance with the local mean, and the presence of outliers all violated the stationarity assumption and strongly hindered the modeling of the spatial structure. Cross-validation tests showed that kriging estimates were better when interpolating within very local neighborhoods using a small number of points. Kriging always performed better than polygonal tessellation. A stratification scheme produced better estimations than the whole-region approach using traditional or relative variograms. The spatial organization of the shrimp biomass was composed of a trend superimposed onto mesoscale patches of 30–50 km in diameter. The area under study contained about 22 000 tonnes of northern shrimp; 70% of this biomass was concentrated in less than 30% of its surface. The spatial information is used to derive guidelines for optimizing future sampling programs.

La méthodologie pour la cartographie et l'estimation globale et par seuil d'abondance de ressources exploitables autocorrelées est présentée, suivant des méthodes géostatistiques stationnaires. L'usage et la performance de ces méthodes en écologie marine sont illustrés par une application à l'abondance de crevette nordique (*Pandalus borealis*), échantillonnée à 137 stations dans l'ouest du golfe du Saint-Laurent en 1989. La non-stationnarité de la biomasse, un accroissement de la variance locale proportionnel à la moyenne locale et la présence de valeurs extrêmes violaient tous l'hypothèse de stationnarité, et rendaient difficile la modélisation de la structure spatiale. Des tests de validation croisée ont montré que les estimés de krigeage étaient meilleurs en restreignant l'interpolation à un voisinage très local et en utilisant peu de points. Le krigeage a toujours montré une meilleure performance que la méthode de tessellation polygonale. Les estimations suivant une stratification de la région étaient meilleures que celles sans stratification utilisant un variogramme traditionnel ou relatif. L'organisation spatiale de la crevette montrait une tendance superposée à des agrégations de 30–50 km de diamètre. La région à l'étude contenait environ 22 000 tonnes de crevette nordique; 70% de cette biomasse était concentrée dans moins de 30 % de sa superficie. Les lignes directrices pour l'optimisation de programmes d'échantillonnage futurs sont déduites de l'information structurale.

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Physical, chemical, and biological variables in natural environments often present well-defined structures in time and space, such as gradients, recurrent patches, mosaics, and other complex patterns. These structures, which can be described statistically (Legendre and Fortin 1989), are intrinsic characteristics of ecosystems. They are generated by complex spatio-temporal processes acting over a continuum of scales (Margalef 1979), like the various mechanisms controlling the distribution of chemical compounds in soils (Yost et al. 1982; Burgess and Webster 1980a, 1980b), the assemblages of plant species in forests (Marbeau 1976; Legendre and Fortin 1989), or the organization of herbivores and their predators. In aquatic environments, currents, water masses, nutrients, plankton, fish, and whales are also not distributed at random but well organized in time and space (Haury et al. 1978; Steele 1978; Margalef 1979; Mackas et al. 1985; Legendre and Troussellier 1988).

In this situation, the realization of a variable at one location is dependent on its realization at nearby locations. This spatial autocorrelation requires special considerations in estimating these variables. Commonly used classical estimation methods assume the independent selection of the samples, which allows the application of classical sampling designs dictating the

(rather rigid) distribution of the samples through space (e.g. Cochran 1977). Classical estimation methods cannot be used when the data have not been obtained following these sampling designs.

When the spatial structure is known and stable through time, the space is often discretized into homogeneous strata, where spatial structuring is assumed to be absent or negligible. Allocation of the samples may then be optimized by stratification in order to minimize the variance of the estimate and the structure-dependent bias. The precision of the mean is strongly dependent on the effectiveness of the strata definition, the allocation of samples to strata, and on the stability of the known structure (Cochran 1977). When the spatial structure is unknown or unstable through time, this method will not always reduce the variance of the estimate. Such situations are common in aquatic environments.

A third and major problem lies in the fact that the formulas of classical statistics used to compute the confidence interval of the mean of the resource are based on estimates of the variance which, in turn, assume that the error terms of the samples are stochastically independent of one another. This condition is not met by spatially autocorrelated data (Cliff and Ord 1981) so that variances and confidence intervals calculated from these classical formulas are unrealistic. Geostatistics (Matheron 1971) tells us that, besides the shape of the spatial autocorrelation and the density of samples, the variance of an estimate also depends on (1) the geometry of the volume to be estimated, (2) the spatial organization of the set of samples, through the relative location of samples, and (3) the location of the samples in the volume.

Geostatistics was developed to deal with estimation problems in spatially autocorrelated phenomena. It makes use of the additional information provided by autocorrelation when computing the estimates and their confidence intervals. It has proved useful in various disciplines such as geology, forestry, hydrology, oceanography, etc., and its application is presently extended to the estimation of exploited marine resources, where classical methods present difficulties (Conan 1985; Nicolajsen and Conan 1987; Armstrong et al. 1989; Petitgas and Poulard 1989). The general applicability of geostatistics for this latter task is, however, questionable. It assumes that the probability law describing the spatial structure is known or can be adequately modeled from a limited set of samples. This is not obvious for standard data sets in marine ecology, where trends and complex spatial structures are common, and where the number of samples is generally low compared with the high variability present. Besides, the theory of geostatistics is well described but its practice is much less documented (Isaaks and Srivastava 1989), and detailed applications for various tasks in marine ecology are lacking. The present paper explores geostatistical methods to analyze the spatial structure and estimate the biomass of the northern shrimp (*Pandalus borealis*) in the western Gulf of St. Lawrence in the fall of 1989. The reason for using geostatistics with the present data is twofold. First, our data are autocorrelated, so that a correct estimation of the confidence interval of the mean could not have been obtained from classical statistics. This confidence interval will be necessary to assess whether a significant temporal trend is present in the evolution of the stock. Second, sampling has not been carried out following one of the classical sampling plans (random, regular, stratified, and so on) and comes from three different concurrent research projects.

The exploited biomass of the northern shrimp in the Gulf of St. Lawrence is found on muddy bottoms between depths of 150 and 350 m (Fig. 1). It is spatially autocorrelated. For the present data, the value of the generalized Moran's I statistic (Moran 1950; Cliff and Ord 1981) that can be computed for the various distance classes is 0.32 for the distance class of 0–30 m, which is significantly different from zero ($p < 0.001$). The spatial organization presents aggregations in various areas that are sought and exploited by the fishermen. In the western Gulf of St. Lawrence, the northern fringe of the Laurentian Channel, between depths of 150 and 250 m (Fig. 1), tends to consistently support higher densities of shrimp. Except for this general pattern and for the transient congregation of berried females on the shallow bottoms around 150 m in winter and spring in some ares (Savard 1989), the aggregations show high, seasonal and interannual, unpredictable variations (Y. Simard, unpubl. manuscr.). Despite their primary importance for the industry and the fishermen, little is known on the spatial structure of the shrimp biomass in the Gulf or on the mechanisms that control its variability.

Changing spatial structures profoundly hinder the direct estimation of the global biomass on fishing grounds, for all sampling plans. When using a stratified random plan (Cochran 1977; Mackett 1973), the determination of effective strata is difficult because the location of the aggregations is unstable, and unpredictable without a priori knowledge of the existing structure. We therefore need a method of estimation that is tolerant to the geographic organization of the biomass, such as tessellation methods of interpolation (like Delaunay's or Dirichlet's), spline approximation, or kriging (e.g. Conan 1985; Stolyarenko 1986; Smith and Mohn 1987; Isaaks and Srivastava 1989).

An additional advantage of geostatistics is its capacity of producing not only global estimates of the total biomass in the study area but also local and cutoff estimates, with their confidence intervals, in presence of spatial autocorrelation. The cutoff estimates correspond to the total biomass incorporated in the delineated area where the biomass exceeds given threshold values. This information is desirable in the estimation of exploitable natural resources whose profitability of exploitation not only depends on the total stock but also on its distribution. The exploitable biomass is given by the cutoff estimate associated with the locations where shrimp biomass is high enough to be fished profitably. The area over which this biomass fraction is distributed and the distribution pattern are also interesting measurements because they directly influence the level of financial profit. Other potentiality of geostatistics is the use of the structure function to optimize sampling strategies (e.g. Burgess et al. 1981) and to draw inferences on the process generating the aggregations (Sokal 1986).

This paper will address the basis of kriging methodology and its application, point out its limitations, and use it to (1) compute optimal estimates of the global biomass, with their confidence intervals, (2) objectively map this biomass, describe the spatial patterns and compute cutoff estimates of the exploitable proportion above given threshold densities, and (3) analyze the structure and exploit its information to optimize sampling strategies.

Methods

Sampling

From 31 August to 10 October 1989, northern shrimp biomass was sampled at 137 stations in the western Gulf of St.

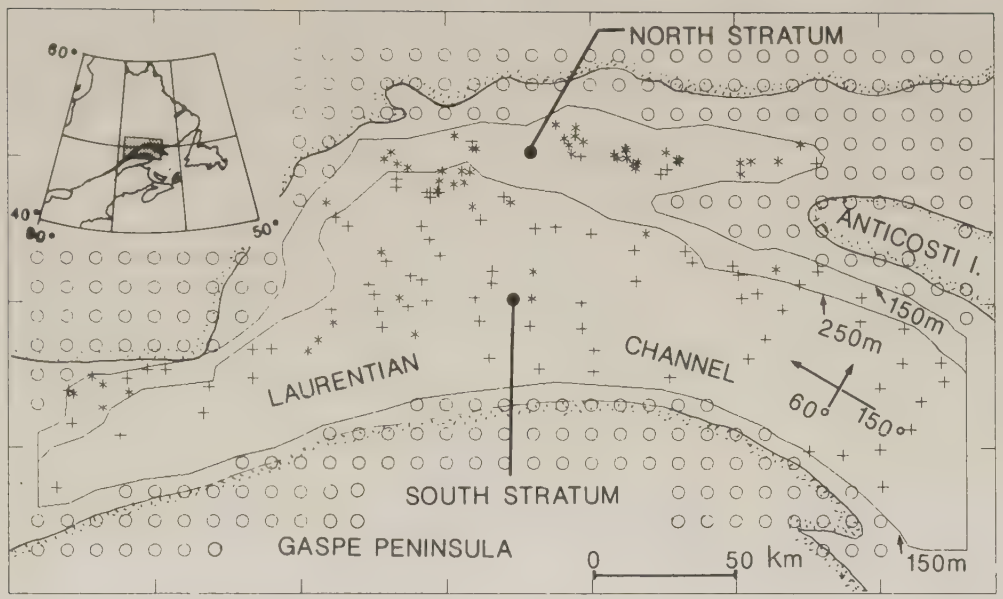

FIG. 1. Map of the western Gulf of St. Lawrence with sample locations and the contour of the studied region at depths greater than 150 m. The isobath of 250 m on the north separates the two strata, north and south. *, >1000 kg shrimp/km^2; +, <1000 kg shrimp/km^2; ○, imposed zeros at the nodes of the interpolation grid outside the studied region for guiding contours (see text).

Lawrence (Fig. 1) by the trawlers M.V. *Bradelle* (26.5 m) and F.R.V. *Alfred Needler* (50 m). The *Bradelle* visited most (120) of the stations and 16 of them, located in the richest area, were paired by the *Alfred Needler* which sampled simultaneously at a nearby station. At each station, bottom shrimp trawls were towed for 30 min at 2.5–3.0 knots. The *Bradelle* used a Western II-A trawl and the *Alfred Needler* used a URI 81/114 trawl. The stretched mesh aperture was 38 mm and the codend was lined with a 19-mm mesh. Since the *Alfred Needler* caught significantly (Wilcoxon test, $p < 0.05$) more shrimp (17% on average) than the *Bradelle*, because of her more efficient trawl, her catches per square kilometre were converted to *Bradelle* catches by means of a linear regression ($r^2 = 0.82$). All sampling was carried out during daytime because vertical migrations of the shrimp off the bottom at night (Barr 1970; Apollonio et al. 1986) could change their availability to the gear. Because of shrimp availability to the gear, avoidance of the gear, and trawl sampling efficiency, the catch does not represent 100% of the shrimp present. Since this is neglected here, the computed estimates of shrimp biomass are conservative. Although the samples covered an approximate area of 14.5 m per about 2.5 km (≈0.035 km^2), they were considered as point samples centered at the starting tow coordinates.

Geostatistical Model

The local and global estimations computed here were obtained using the usual ordinary punctual kriging technique of stationary geostatistics, briefly outlined below; for complete presentations, see Clark (1979) or Isaaks and Srivastava (1989).

Let the shrimp biomass, SHR, at n locations of the sampled region be described by the series of random variables SHR(x_1), SHR(x_2),. . .SHR(x_p),. . .SHR(x_n). Since the shrimp biomass is spatially autocorrelated, the random variables are linked together by a random function, $Z(x)$, which describes the dependence of the random variables SHR(x) on each other. The characteristics of this model can be estimated from the sample data set, if we assume some stationarity conditions.

Stationarity

In the case of ordinary kriging (i.e. the global mean is unknown), the following second-order stationarity conditions are assumed:

(1) $E[Z(x + h) - Z(X)] = 0$

(2) $\mathrm{VAR}[Z(x + h) - Z(x)] = 2\gamma(h)$

where h is the distance separation between two locations (which can have a directional component) and the function $\gamma(h)$ is the semi-variogram (commonly called the variogram). These two stationarity conditions are referred to as the "intrinsic hypothesis." In this situation, the *difference* in $Z(x)$ does not depend on the locations x but only on the geographic distance separation h, and the average of this difference over the studied region must be zero. These stationarity conditions ask for a certain degree of regional homogeneity of the shrimp biomass and for absence of trend.

These conditions must be carefully checked for proper estimation through stationary geostatistics. Spatial trends in data sets must be removed prior to the analysis and special consideration given to outliers in data sets and to highly skewed probability distributions (e.g. lognormal), which greatly affects the estimation and interpretation of the variogram. Normal distributions are also more prone to provide kriging estimates close to conditional expectations and kriging errors that are normally distributed. For lognormal distributions, normalization of the data by transformation to logarithmic units and the use of the lognormal kriging technique can help minimize problems (Rendu 1979; Journel 1980). Results are, however, very sensitive to slight departures from the strict lognormal distribution and to the choice of the variogram model, and therefore this method must be used with caution (Armstrong and Boufassa 1988). Another way to minimize the effect of increasing variance with the mean is to compute a relative semi-variogram,

$\gamma(h)$/mean^2, using the semi-variogram of the log-transformed data, which is then back-transformed to the raw units according to Journel and Huijbregts (1978).

An implicit assumption of geostatistics is that the structure is stable in time, i.e. the spatial organization of the biomass is fixed, at least for the duration of the sampling period. This constraint might be important for highly mobile species but it is not very restrictive for shrimp. Even though they are not sessile animals, the displacement of their biomass is rather slow compared with the survey speed. Another underlying assumption is that the capturability by the sampling gear is not structured through space. This is likely to happen when the sampling grid is visited around the clock and day and night stations are mixed together, because of diel vertical migrations of the shrimps. This was minimized here by sampling only during daytime, when shrimp are more fully available to bottom trawls than during the night.

Local estimation

Local estimation[1] of the biomass $Z(x_0)$ at a point x_0 is done by interpolation according to the following equation using the data points $Z(x_i)$ available in the surrounding neighborhood chosen:

$$(3)\quad Z^*(x_0) = \sum_{i=1}^{n} w_i \cdot Z(x_i)$$

where w_i are weights, which sum to 1 to insure that the estimate is unbiased ($E[Z^*(x_0) - Z(x_0)] = 0$). The w_i are estimated in such a way that they minimize the variance of error of the estimate ($\sigma_k^{*2}(x_0)$), called the kriging variance (VAR$[Z^*(x_0) - Z(x_0)]$ = minimum). The solution to minimize $\sigma_k^{*2}(x_0)$ is obtained by partial differentiation relative to the weights w_i, taking into account that they must sum to 1 (Isaaks and Srivastava 1989). In matrix notation:

$$(4)\quad \boldsymbol{C} \cdot \boldsymbol{W} = \boldsymbol{U}$$

$$\begin{bmatrix} C^*_{11} & \cdots & C^*_{1n} & 1 \\ \vdots & & \vdots & \vdots \\ C^*_{n1} & \cdots & C^*_{nn} & 1 \\ 1 & \cdots & 1 & 0 \end{bmatrix} \cdot \begin{bmatrix} w_i \\ \vdots \\ w_n \\ \mu \end{bmatrix} = \begin{bmatrix} C^*_{10} \\ \vdots \\ C^*_{n0} \\ 1 \end{bmatrix}$$

where the C^*_{ij} are the covariances between the random variables corresponding to the samples ($C^*_{ij} = E\{[Z(x_i) - E(Z(x_i))] \cdot [Z(x_j) - E(Z(x_j))]\}$) that describe the spatial structure of the probabilistic model. The Lagrange parameter, μ, is a mathematical artifice introduced to constrain the weights to sum to 1. The C^*_{ij} are calculated by means of a function that expresses the covariance in terms of the distance separation between samples $C^*(h) = \gamma^*(\infty) - \gamma^*(h)$ where $\gamma^*(h)$ is the estimated variogram and $\gamma^*(\infty)$ is its sill. This sill is equal to the variance of the population (σ^2) under the assumed stationarity conditions, when the sampled field is larger than the spatial structure. Since $\gamma^*(\infty)$ is a constant, the covariance terms, C^*, in equation (4) are replaced by the variograms γ^* without changing the equality. The weights are found by solving $\boldsymbol{W} = \boldsymbol{C}^{-1} \cdot \boldsymbol{U}$, and they are substituted in equation (3) to estimate $Z^*(x_0)$. The corresponding minimized error variance of this estimate, $\sigma_k^{*2}(x_0)$, is obtained from the following matrix equation:

$$(5)\quad \sigma_k^{*2}(x_0) = \sigma^{*2} - \boldsymbol{W} \cdot \boldsymbol{U}$$

[1]The asterisk distinguishes estimates from true values.

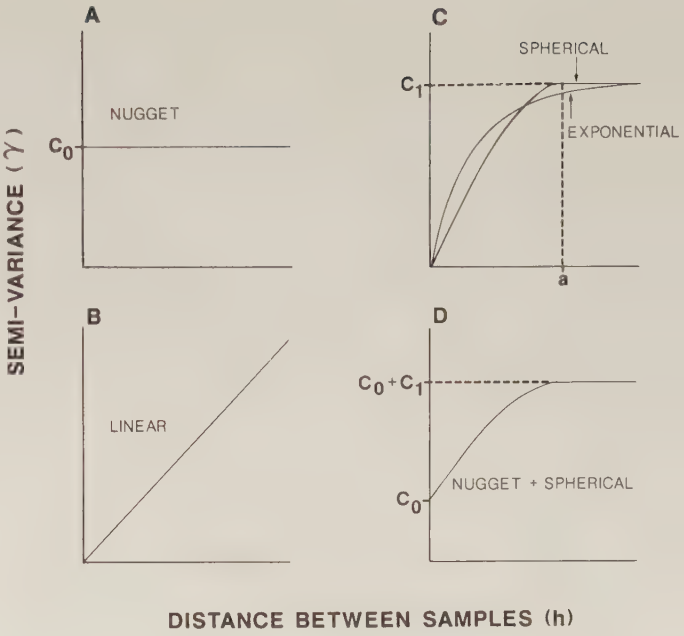

FIG. 2. Common variogram models. (A) Nugget model, $\gamma(h) = C_0$; (B) linear model, $\gamma(h) = bh$; (C) spherical model, $\gamma(h) = C_1 [3h/2a - h^3/2a^3]$ if $h \leq a$ and $\gamma(h) = C_1$ if $h > a$, where a is the range of autocorrelation and C_1 is the plateau of semi-variance or the sill; and exponential model, $\gamma(h) = C_1[1 - \exp(-h/a)]$; (D) nested nugget + spherical model, $\gamma(h) = C_0 + C_1 [3h/2a - h^3/2a^3]$ if $h \leq a$ and $\gamma(h) = C_0 + C_1$ if $h > a$.

where σ^{*2} is the variance of the samples. When $\boldsymbol{W}$ and $\boldsymbol{U}$ are obtained from a relative semi-variogram, the computed σ_k^{*2} must be multiplied by the kriged estimate squared, Z^{*2}, to obtain the absolute minimized error. Kriging is referred to as the best linear unbiased estimator (BLUE). It is also an exact interpolator: at a sampled location, x_p, the interpolation always passes through the data point (i.e. $w_p = 1$ and all other $w_i = 0$). This is true even if the variogram does not pass through the origin (nugget effect, see below); the interpolation surface is then discontinuous at the data point.

In practice, local estimates are computed using only the nearest samples included in a confined neighborhood. The shape and size of this neighborhood and the data points retained for the estimation affect the value of the estimate. They are chosen according to the model of spatial continuity and to the distribution of samples in the area (Isaaks and Srivastava 1989). Commonly used variogram functions are the *nugget model*, which corresponds to the absence of a spatial structure (random spatial organization) (Fig. 2), the *linear model*, the *spherical model*, and the *exponential model*, whose sill, C_1, indicates the maximum variability due to the structure and whose range of autocorrelation, a, gives the distance at which the samples are no longer autocorrelated. Combinations of these functions are also used to model nested structures, which often include a nugget model to represent the unresolved small-scale variability and sampling error (C_0). The ratio $C_0/C_0 + C_1$, where C_1 is the variability due to the structure in models with a sill, gives the proportion of this latter unresolved random variability to the total variability ($C_0 + C_1$). Optimal choices of variogram and kriging neighborhood are often developed using a jackknife cross-validation method. In this procedure, the data points are removed one by one and are estimated using the chosen variogram model and kriging parameters. The best solution is the

one which, among other properties, minimizes the mean square residuals, produces the standardized residuals ($[Z^*(x_i) - Z(x_i)] / \sigma_k^{*2}(x_i)$) the closest to the conditional expectations of a mean of 0 and a standard deviation (SD) of 1.0, and maximizes the correlation coefficient between the estimated and the observed values, whose linear regression should have a slope close to 1.0 (Samper Calvete and Neuman 1989).

Global and cutoff estimations

Global estimation of the mean biomass density (Z^*) is easily obtained by averaging the m local estimates, computed at the nodes of a regular grid covering the studied region. The total biomass of shrimp (ZD*) is then estimated by multiplying this mean density (grams per square metre) by the surface area S_D of region D:

$$(6) \quad ZD^* = S_D \cdot \frac{1}{m} \cdot \sum_{j=1}^{m} Z^*(x_j).$$

Three-dimensional numerical integration of the mapped biomass of shrimp can also be used for this computation. Cutoff estimates corresponding to the area where the biomass exceeds a given threshold are obtained similarly, using only the local estimates above the chosen biomass threshold.

The variance of these estimations is much more difficult to estimate because we have only one realization of ZD. Two possibilities using the extension variance approach are formulated below. The first procedure computes the variance of the global estimation by summing the variance of strata forming a mosaic over the whole region (Petitgas and Poulard 1989). The strata variances are assumed to be independent of each other. The size of the strata must be smaller than the range of reliability of the computed experimental variogram, which is always less than half the dimension of the studied region (Journel and Huijbregts 1978). The variance σ_D^2, of ZD is then

$$(7) \quad \sigma_D^2 = \sum_l \sigma_l^2 S_l^2$$

where

$$(8) \quad \sigma_l^2 = 2\bar{\gamma}(x_i, S_l) - \bar{\gamma}(S_l, S_l) - \bar{\gamma}(x_i, x_j)$$

where S_l are the surface area of the stratum l, $\bar{\gamma}(x_i, S_l)$ is the average semi-variance between the particular sample locations i in the stratum l and all the points of the stratum (which is an averaged point-to-stratum semi-variance), and $\bar{\gamma}(S_l, S_l)$ is the equivalent for all possible pairs of points in the stratum and $\bar{\gamma}(x_i, x_j)$ for all pairs of samples in the stratum. These average semi-variances $\bar{\gamma}$ are computed from the experimental point-to-point variogram. To estimate the point-to-stratum and the stratum average semi-variances, the strata are discretized in a grid of points and the average semi-variance is estimated using the point-to-point variogram. This variogram can be unique for the whole region when global stationarity holds, or it can be computed for each set of homogeneous strata when it does not. This computation of the confidence intervals of the global estimate is time consuming and awkward when the number of strata and samples is large.

The second procedure is the combination of the elementary sampling errors, which computes the variance of the global estimation by the summation of elementary independent errors (Journel and Huijbregts 1978). Each sample x_i is assigned a surface of influence s_i. The error made when the value of the central point sample Z_{xi} is extended to represent the values of all points in the surfaces x_i is computed according to the formula

$$(9) \quad \sigma_{Esi}^2 = 2\bar{\gamma}(x_i, s_i) - \bar{\gamma}(s_i, s_i) - \bar{\gamma}(x_i, x_i)$$

where $\bar{\gamma}(x_i, x_i) = 0$. The weighted sum of these elementary errors is the variance of the global estimate of the total biomass:

$$(10) \quad \sigma_D^2 = \sum_i s_i^2 \sigma_{Esi}^2.$$

These calculations are cumbersome when the number of samples is large, since they require the computation of the surface of influence of all samples and the estimation of each error variance. An easier approximation of σ_D^2, when the sampling density is uniform, can be obtained by computing the extension variance of a random point in the average elementary surface s, whose dimensions must respect the length/width ratio of the region D. This is given by the formula

$$(11) \quad \sigma_D^2 = \bar{\gamma}(s, s) \cdot s^2 \cdot N$$

whose solution is directly obtained from geostatistical charts (Journel and Huijbregts 1978).

The variance of cutoff estimations is obtained similarly except that the variogram used is the one computed using only the samples where the shrimp density exceeds the given cutoff (Froideveaux 1984).

In our example, we have used a grid spacing of 10 × 10 km, which roughly corresponds to the average spacing between the data points. The contour line of the stock was limited to depths greater than 150 m, the grounds occupied by adults of *Pandalus borealis* in the Gulf of St. Lawrence. Zeros were imposed at the nodes of the grid in shallower bottoms outside this isobath and on land (Fig. 1), and they were used only to force the contours of biomass levels to follow the contour of the sampled area. They were not used as information points during the interpolation process because we have found that the biomass contours obtained in this way were constrained unrealistically.

The similarity between the interpolation grids obtained by kriging was measured using the Pearson correlation coefficient. Since all these maps were obtained from the same set of 137 observations, the kriged maps are not independent of one another and thus correlation coefficients cannot be tested for significance. They remain, however, a valid measure of resemblance. If they were coming from independent data sets, corrections would still have to be introduced in the testing procedure to account for the lack of independence (spatial autocorrelation) of the data points (Clifford et al. 1989).

Interpolation and mapping by *polygonal tessellation* was also used. This method simply consists of attributing the value of the nearest sample to the estimated location, which results in a mosaic of tiles whose surfaces are the surface of influence of each sample (Isaaks and Srivastava 1989). For estimating the global mean and variance, the samples are then weighed by their surface of influence.

Results

Stationarity Conditions

The biomass of shrimp per square kilometre was not homogeneously distributed and did not show a monotonous trend that could easily be modeled. Two areas of different density were found. As expected, the northern side of the Laurentian Channel was distinctly richer than the rest of the surveyed region (Fig. 1 and 3). Because this large-scale structure violated the stationarity conditions, the region was divided into two strata, north and south, bounded by the

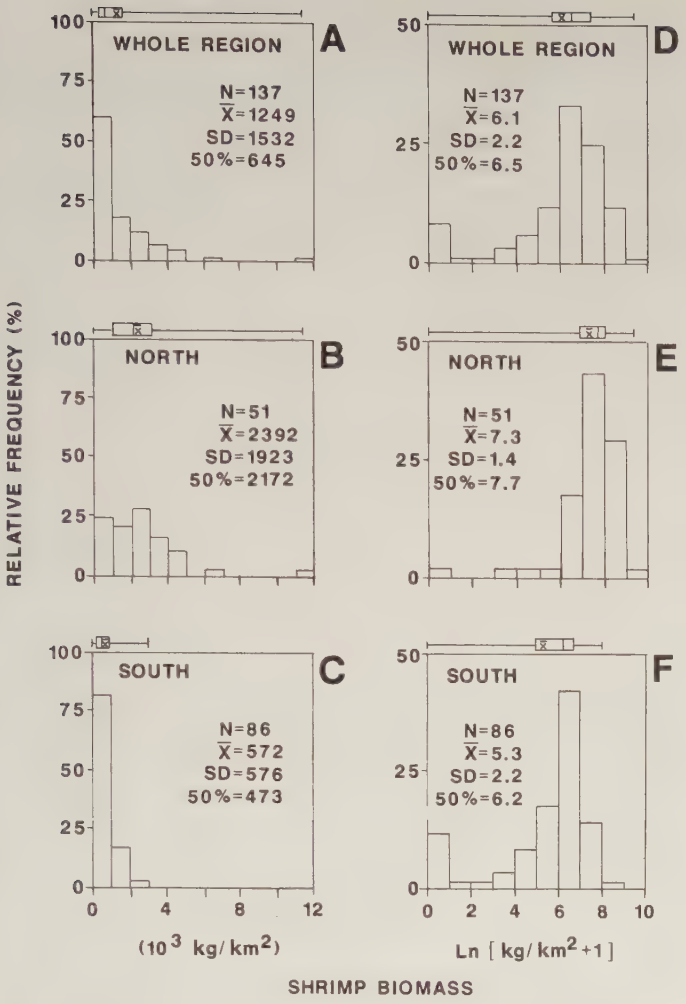

FIG. 3. Histograms with box plots and statistics of the shrimp biomass samples for the whole region and for the north and south stratum separately, for (A–C) raw and (D–F) log-transformed data.

150-m contour towards the coast and separated by the 250-m contour on the northern side of the channel (Fig. 1).

In the whole region and in both strata, shrimp were contageously distributed, and probability distributions were significantly skewed (Fig. 3). The Box–Cox procedure (Box and Cox 1964; Sokal and Rohlf 1981) to identify the best normalizing transformation gave an estimated lambda of 0.30 (95% C.I. = [0.24, 0.37]), indicating that the shrimp biomass was far from the lognormal distribution (lambda = 0), thus precluding the use of lognormal kriging (Armstrong and Boufassa 1988). Because kriging using Box–Cox transformations is not yet described and because transformations are delicate for resource estimation (David 1988), we have only used kriging on the original data. Back-transformation in the original metric often leads to substantial bias when the transformation used and/or the structure model are improper, even only slightly.

One outlier, one order of magnitude higher than the mean, was encountered in the western end of the region. To minimize the effect of nonstationarity, indicated by the increasing variance with the mean, kriging was also performed using a relative variogram computed for the whole region. A third estimation using a normal variogram for the whole region was computed for comparison.

Variograms and Spatial Structure

The omnidirectional variogram of the shrimp biomass of the north stratum was relatively erratic (Fig. 4A). Except for the initial point at an average distance of 3 km, all points were dispersed along a line of constant semi-variance of 2 100 000, indicating no spatial autocorrelation of the shrimp biomass at distances larger than 10 or 15 km. A large part of the variability in this stratum was therefore random, and the structure, if any, existed only at small scales. The variogram was tentatively modeled by a nugget effect, representing the unresolved small-scale variability, over which can be superimposed either (1) a weak monotone structure extending over the entire stratum area (Table 1, linear model, not illustrated in Fig. 4A) or (2) a fine structure representing small patches with radius of 10–15 km (Table 1, spherical model with a range of 15 km, Fig. 4A). In this latter mixed model, the variability due to the structure (C_1 = 1 600 000) was about three times higher than the unresolved small-scale variability (C_0 = 500 000). The variogram in the east–west direction (not shown) did not significantly differ from the omnidirectional one. The distribution of the stations in this stratum precluded the computation of significant variograms in other directions. Isotropy was therefore assumed and the isotropic nugget + spherical variogram (Table 1, underlined) was used for the estimations.

The omnidirectional variogram of the south stratum (Fig. 4B, circles) presented a maximum semi-variance much lower than the richer north stratum (Fig. 3). The steady increase of semi-variance with distance indicated that a trend was present. Significant anisotropy was also observed, with the directional variograms across (direction 60°) and along (direction 150°) the Laurentian Channel being different. The low semi-variance at the smallest distance classes on the omnidirectional variogram indicated that small-scale structure could be nested within the large-scale gradient. Various models reasonably fit the variograms (Table 1) and, despite the apparent anisotropy, the two-structure isotropic model consisting of a spherical model *plus* a linear model *plus* a nugget effect performed as well as the anisotropic models and was used for the kriging estimation (Table 1, underlined). The unresolved small-scale variability (C_0 = 60 000) represented a maximum of about 20% of the larger-scale variability due to the structure.

The omnidirectional variogram computed for the whole region (Fig. 4C) gave a rough average of the variograms of the two strata. Its shape was dominated by a large-scale structure with a radius of about 110 km. It was easily modeled by a spherical model with a range of 110 km superimposed over a high nugget effect (C_0 = 750 000), representing an unresolved small-scale variability corresponding to 65% of the structural variability (C_1 = 1 150 000).

The relative variogram was characterized by a plateau of semi-variance from about 20 to 60 km, followed by an increase of semi-variance at larger distances (Fig. 4D). Neglecting the large-scale variability, a spherical model was added to a nugget effect to model the small-scale semi-variance (Table 1), and this variogram was used for kriging.

Cross-validation

All the structures modeled performed poorly in estimating the true value of the samples, especially when a large search radius and a high number of points were used (Table 1). Most solutions reasonably satisfied the global unbiasness condition (mean of standardized residuals = 0) but the distributions of

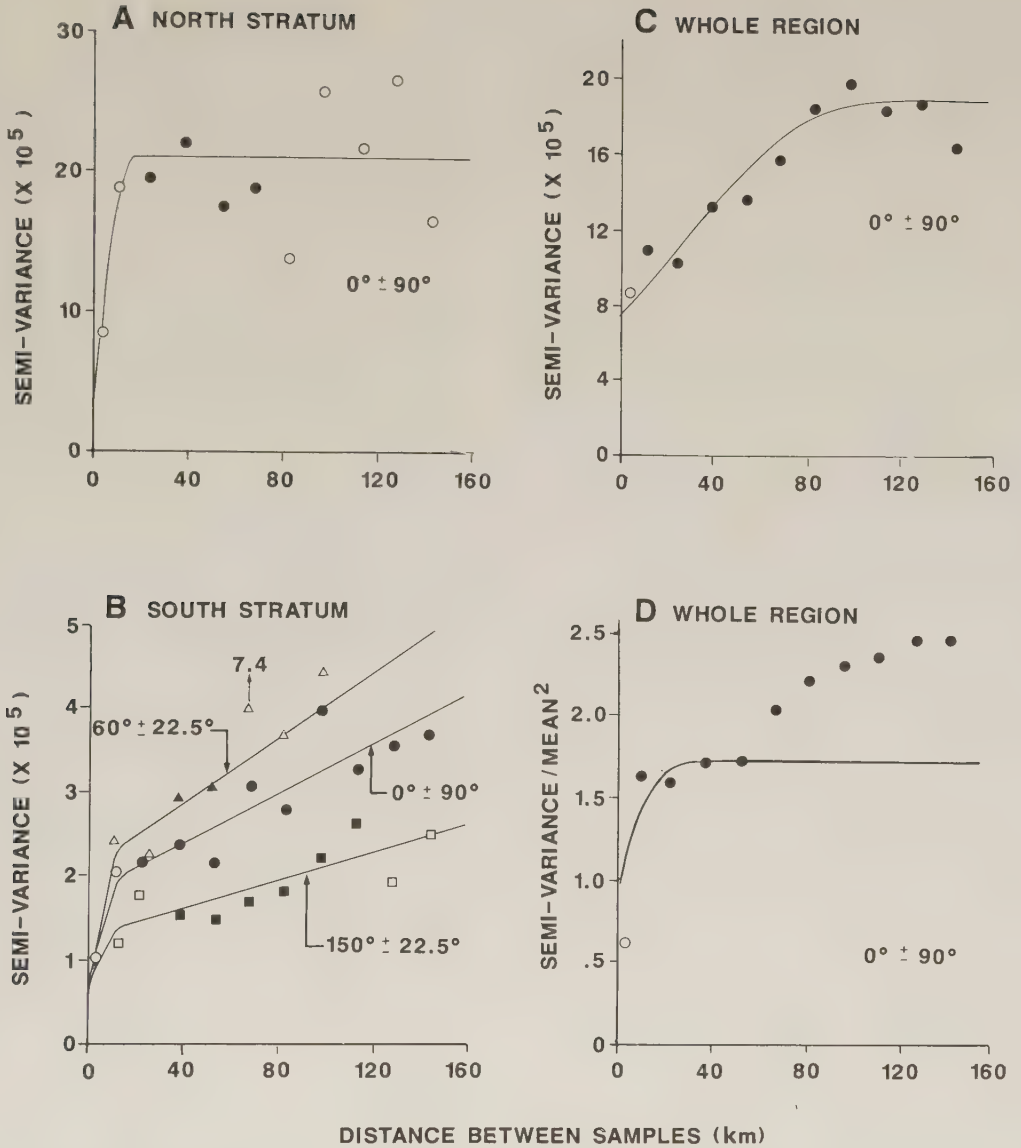

FIG. 4. Variograms computed for (A and B) the two strata separately and (C and D) the whole region. Isotropic variograms (0° ± 90°, circles) and directional variograms along and across the Laurentian Channel (60° ± 22.5°, triangles; 150° ± 22.5°, squares). Fitted models are presented in Table 1 (underlined). Except for the relative variogram (Fig. 4D), all variograms exclude the outlier value of 11 373 kg/km^2. All points include more than 100 pairs, except open symbols (20–100 pairs).

the errors of the estimates, as indicated by their SD's, varied notably from one another. This latter parameter, together with the correlation coefficients of the true values against the estimates, was used to select locally optimal solutions. The best estimations of the samples were obtained using either the two or the four nearest samples, for all the variogram models explored (Table 1, underlined). The goodness of fit was, however, weak with less than 50% of the variance being explained by the chosen spatial models.

The slopes of the regressions of the true values against the estimates were very different when computed with raw or log-transformed data. This resulted from the fact that the variance of the raw data increased with the value of the estimates. The logarithmic transformation maximized the homoscedasticity condition for the regression by giving less weight to the high values (for which the error is high) which more greatly affected the slopes. For all models, the slopes of the log-transformed data converged towards the value of 1 when the correlation coefficients were maximum; these trials were thus chosen as the optimal ones. The corresponding slopes for the raw data were smaller than 1, indicating that, on the average, the kriging estimates underestimated the low values and overestimated the high values (''conditional bias'': Isaaks and Srivastava 1989). Examination of the residuals of the regressions showed that this conditional bias of the optimal models chosen was small, and mainly resulted from large errors associated with a few high values.

Interpolation by polygonal tessellation did not perform well in the cross-validation tests (Table 1). The slope parameters were always far from 1 and the SD's of the errors of the estimates were larger than the kriging alternatives.

In the north stratum, the cross-validation clearly showed that the linear model with high nugget effect was unsuitable. The estimates were uncorrelated with the true values, except when only two points were used. Lowering the nugget parameter by fitting a small-scale structure using a spherical model with a

TABLE 1. Cross-validation of the variograms models and search methods. SD of the errors of the estimates (SD_E) from the true value ($Z^*(x_i) - Z(x_i)$) obtained using the given variogram and the search method at the sample points (x_i); Pearson correlation coefficients (r_1 and r_2) and slopes (b_1 and b_2) of the linear regressions $\ln(Z + 1) = a_1 + b_1 (\ln Z^* + 1)$ and $Z = a_2 + b_2 Z^*$; mean, SD, and maximum values of standardized residuals ($Z^*(x_i) - Z(x_i)/\sigma_k^*(x_i)$). For the relative variogram, these residuals are $[Z^*(x_i) - Z(x_i)]/[\sigma_k^*(x_i) \cdot Z^*(x_i)]$. Underlined are the chosen combinations for computing the kriging estimates of Table 3.

	Variogram	Search radius (km)	Max. no. of points	SD_E	r_1	b_1	r_2	b_2	Standardized residuals: Mean	SD	Max.
North stratum[a]	Isotropic, nugget + linear $\gamma(h) = 1\,500\,000 + 5500\,(h)$	200	20	1484	0.26	2.26	0.00	0.02	0.02	1.14	−3.7
		100	20	1486	0.27	1.95	0.02	0.09	0.01	1.14	−3.7
		50	20	1482	0.29	1.64	0.08	0.25	0.01	1.13	−3.7
		50	8	1489	0.29	1.46	0.10	0.28	0.04	1.12	−3.4
		50	4	1515	0.21	1.00	0.13	0.27	0.14	1.09	−3.2
		50	2	1350	0.67	1.55	0.45	0.62	0.06	0.89	−2.8
		25	8	1533	0.15	0.33	0.11	0.24	0.05	1.13	−3.3
		25	4	1537	0.17	0.36	0.16	0.29	0.11	1.10	−3.2
		25	2	1356	0.55	0.99	0.46	0.61	0.05	0.89	−2.8
	Isotropic, nugget + spherical $\gamma(h) = 500\,000 + 1\,600\,000\ \mathrm{Sph}_{15}(h)$	200	20	1334	0.53	2.97	0.39	0.96	0.08	1.06	−3.7
		100	20	1315	0.65	3.00	0.42	0.94	0.02	1.05	−3.7
		50	20	1301	0.66	2.60	0.44	0.90	0.03	1.04	−3.7
		50	8	1314	0.65	2.51	0.43	0.83	0.05	1.03	−3.6
		50	4	1398	0.49	1.88	0.34	0.61	0.08	1.07	−3.5
		50	2	1313	0.69	1.51	0.49	0.66	0.04	0.96	−3.2
		25	8	1357	0.35	0.74	0.39	0.68	0.04	1.05	−3.6
		25	4	1434	0.32	0.66	0.33	0.52	0.06	1.08	−3.5
		25	2	1319	0.58	1.00	0.49	0.65	0.03	0.96	−3.2
	Polygonal method (estimate = nearest sample)	200	1	1553	0.57	0.46	0.35	0.33	—	—	—
South stratum	Isotropic, nugget + linear $\gamma(h) = 130\,000 + 2200\,(h)$	200	20	531	0.73	1.33	0.41	0.77	0.01	1.23	−6.1
		100	20	531	0.73	1.33	0.41	0.77	0.01	1.23	−6.1
		50	20	544	0.71	1.29	0.37	0.69	0.01	1.25	−6.2
		50	8	536	0.73	1.23	0.41	0.70	0.02	1.22	−6.2
		50	4	507	0.70	0.96	0.50	0.76	0.03	1.12	−6.1
		50	2	516	0.70	0.88	0.52	0.66	−0.00	1.06	−5.5
	Isotropic, nugget + linear + spherical $\gamma(h) = 60\,000 + 1500\,(h) +$ $120\,000\ \mathrm{Sph}_{15}(h)$	200	20	496	0.76	1.67	0.51	0.90	0.01	1.09	−5.4
		100	20	496	0.76	1.67	0.51	0.89	0.01	1.10	−5.4
		50	20	518	0.72	1.32	0.46	0.77	0.01	1.13	−5.6
		50	8	517	0.73	1.23	0.47	0.74	0.02	1.11	−5.6
		50	4	498	0.70	0.97	0.52	0.77	0.02	1.05	−5.5
		50	2	512	0.70	0.88	0.53	0.67	−0.02	1.03	−5.0
	Anisotropic, nugget + linear $\gamma(h) = 130\,000 + 800\,(h_{150°}) +$ $3500\,(h_{60°})$	200	20	539	0.71	1.82	0.38	0.74	0.04	1.26	−6.0
		100	20	539	0.71	1.82	0.38	0.74	0.04	1.26	−6.0
		50	20	550	0.69	1.29	0.35	0.65	0.04	1.28	−6.2
		50	8	539	0.72	1.21	0.40	0.68	0.05	1.23	−6.2
		50	4	514	0.70	0.98	0.49	0.73	0.05	1.14	−6.2
		50	2	514	0.71	0.89	0.52	0.67	−0.01	1.05	−5.6
	Anisotropic, nugget + linear + spherical[b] $\gamma(h) = 60\,000 + 850\,(h_{150°}) +$ $70\,000\ \mathrm{Sph}_{15}(h_{150°}) + 2000\,(h_{60°}) +$ $150\,000\ \mathrm{Sph}_{15}(h_{60°})$	200	20	504	0.60	1.24	0.49	0.85	0.03	1.11	−5.1
		100	20	505	0.60	1.24	0.49	0.85	0.03	1.11	−5.1
		50	20	526	0.69	1.27	0.44	0.72	0.03	1.13	−5.3
		50	8	524	0.72	1.16	0.46	0.70	0.04	1.12	−5.3
		50	4	508	0.70	0.97	0.51	0.73	0.04	1.06	−5.3
		50	2	514	0.71	0.88	0.52	0.66	−0.01	1.02	−4.9
	Polygonal method (estimate = nearest sample)	200	1	605	0.61	0.60	0.47	0.45	—	—	—
Whole region	Isotropic, nugget + spherical[a] $\gamma(h) = 750\,000 +$ $1\,150\,000\ \mathrm{Sph}_{110}(h)$	200	20	957	0.76	1.43	0.66	1.05	−0.00	0.97	−4.7
		100	20	958	0.76	1.42	0.65	1.05	−0.00	0.97	−4.7
		50	20	958	0.76	1.39	0.65	1.05	−0.00	0.97	−4.5
		50	8	982	0.75	1.27	0.63	0.95	0.01	0.99	−4.5
		50	4	974	0.74	1.04	0.65	0.86	0.05	0.94	−4.4
		50	2	914	0.74	0.93	0.71	0.81	0.05	0.82	−3.8
	Relative variogram: isotropic, nugget + spherical $\gamma(h)/\mathrm{mean}^2 = 0.934 +$ $0.782\ \mathrm{Sph}_{27.47}(h)$	200	20	1272	0.75	1.85	0.56	1.00	0.07	0.74	−4.8
		100	20	1272	0.75	1.85	0.56	1.00	0.07	0.74	−4.8
		50	20	1282	0.74	1.42	0.54	0.97	0.03	0.79	−4.8
		50	8	1321	0.73	1.18	0.52	0.82	−0.02	0.92	−5.3
		50	4	1117	0.72	1.01	0.53	0.73	−0.07	1.21	−11.3
		50	2	1361	0.73	0.91	0.56	0.64	−0.31	2.45	−25.8
	Polygonal method (estimate = nearest sample)	200	1	1646	0.67	0.65	0.44	0.43	—	—	—

[a]Excluding the outlier value of 11 373 kg/km^2.
[b]Corresponds to a zonal anisotropy for the spherical model.

range of autocorrelation of 15 km resulted in much better estimations. The SD's of the errors of estimates were reduced by 9% on average. Compared with the polygonal method, the kriging model chosen improved by 15% the SD of the error of the estimates.

The results of the cross-validation of the four kriging solutions explored for the south stratum were less variable than for the north stratum. The best solution chosen had a SD of the errors of the estimates 18% lower than the corresponding SD for the polygonal method.

For the whole region, the relative variogram model did not perform as well as the traditional variogram, the SD's of the residuals being 33% larger on average. Both models were, however, much better than the polygonal method, which had SD's of residuals 47 and 77% greater, respectively.

Estimation and Mapping

Point kriging at the nodes of the 10 × 10 km gid was performed for each of the four optimal combinations of variogram and kriging parameters retained by the cross-validation (Table 1). For the two-strata estimation, the estimates were computed in each stratum by ordinary point kriging using, as information points, not only the samples of the given stratum but all the samples of both strata. This was done to prevent the establishment of an artificial discontinuity at the boundary between the two strata. All the maps of the kriged estimates (e.g. Fig. 5) were very similar, the Pearson statistics computed between them being greater than 0.90; the map obtained by the polygonal estimation method differed from the kriged maps, however, as shown by the lower values of the correlation coefficient (Table 2). The differences between the maps mainly resulted from the different smoothing effect caused by kriging with either two or four points.

By contrast, the maps of the kriging SD's were very different (Fig. 6). Because the computation of the kriging SD's involves the variance of the samples per stratum or for the whole region (equation (5)), the two-strata scheme (Fig. 6A) was clearly discernible compared with the whole-region approach (Fig. 6B). The two-strata scheme produced a lower average error but a larger range of variation compared with the whole-region model. For the relative variogram case, the weighing of the relative kriging SD's by the estimates resulted in a much larger range of errors and in a pattern mirroring the map of the estimates (Fig. 5).

Except for a few square kilometres in its eastern end, all parts of the survey region contained some shrimp according to the kriging performed (Fig. 5). The shrimp stock contour (Fig. 5A) was therefore not determined by the samples but by the external zeros imposed a priori to force the contours to follow the boundaries of the study region. The deepest parts of the Laurentian Channel (Fig. 1) were generally poor, especially easterly (Fig. 5B–5C). The shrimp were concentrated in a long and wide northern patch (Fig. 5B), which exhibited three rich areas (Fig. 5D), and in an isolated 40-km patch in the western end of the survey region (Fig. 5B–5D). The exploitable areas, with densities higher than 1000 kg/km^2 (Fig. 5C), covered 27–30% of the surface of the whole region and they contained slightly more than 70% of the total biomass (Fig. 5C and 5D; Table 3). Areas richer than 1500 kg/km^2 represented about 60% of the total biomass, concentrated in 20–22% of the total surface. A global biomass of about 22 kt was present in the region during the sampling period (Table 3), according to the kriging estimations. The polygonal method gave a global estimate 14%

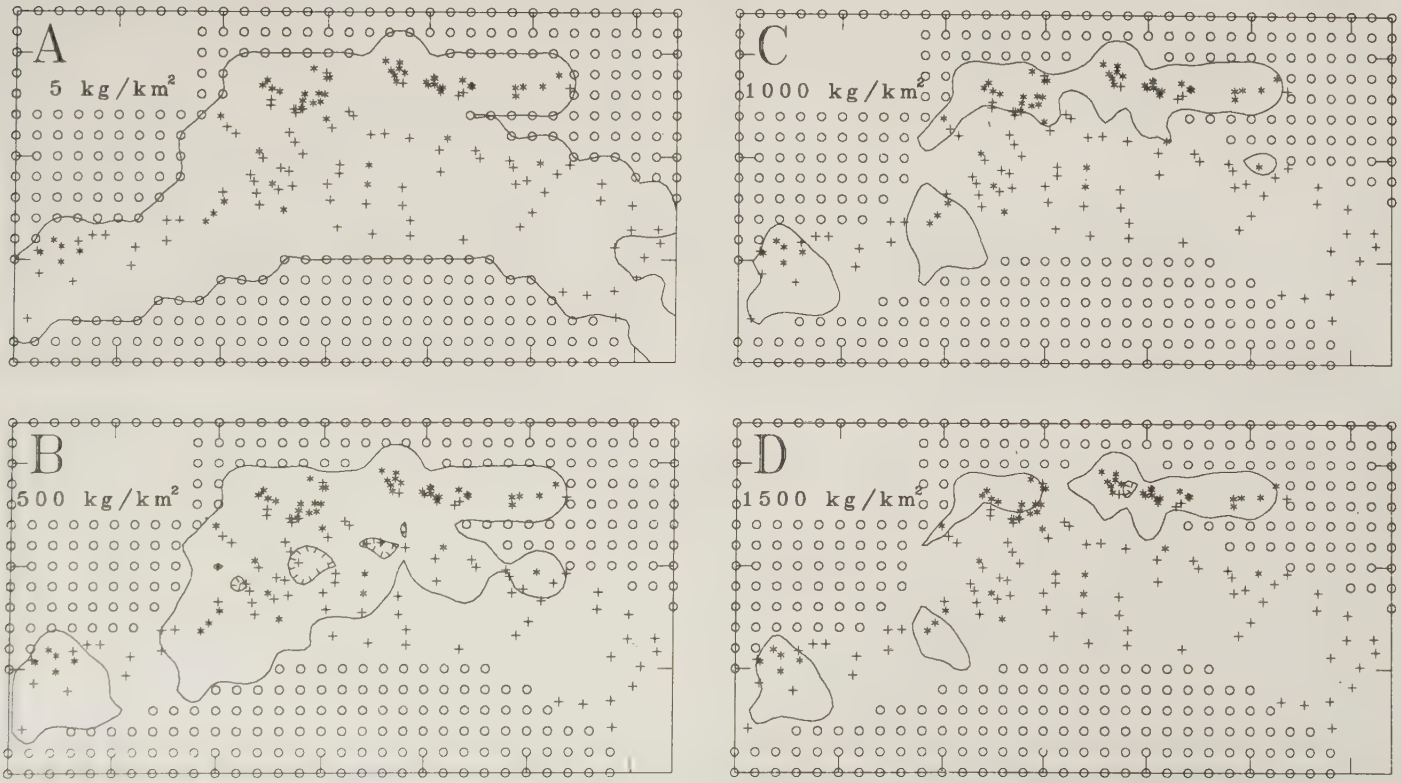

FIG. 5. Maps of the shrimp biomass contours of 5, 500, 1000, and 1500 kg/km^2 obtained from ordinary point kriging using the two-strata model presented in Table 1. Hatched contours are holes. Symbols as in Fig. 1.

TABLE 2. Comparisons of the maps of the shrimp biomass computed using the combinations of kriging parameters chosen in Table 1. Resemblance from the Pearson correlation coefficients computed between the kriged estimates of the maps. These coefficients cannot be tested (see text).

Method	Two strata	Whole region	
		Traditional variogram	Relative variogram
Polygonal tessellation	0.86	0.83	0.78
Whole-region relative variogram	0.95	0.90	
Whole-region traditional variogram	0.92		

lower. The estimated relative standard errors (SE = σ_D/total biomass, σ_D computed from equation (11)) of the kriging global estimates were one third to one half lower than the SE's of the polygonal estimate, and the two-strata model produced the lowest error. The SE's of the cutoff estimates were relatively low.

Discussion

Biomass Structure

Inferences on the spatial structure from the variograms must be done with caution because stationarity conditions were not satisfied. Since the variance tended to increase with the mean and the variograms differed in the range and shapes of the autocorrelation function depending on the area considered, the direction of the variogram, and the type of data transformation, one should concentrate on the local level and on each stratum separately.

At the smallest scale of the observation window, the unresolved variability was not negligible and it increased with the mean biomass of the strata, meaning that either the sampling error or the amplitude of small spatial patterns of biomass, or both, increased with shrimp density. Sampling errors are likely to increase with shrimp density because of the proportional errors associated with the effective area sampled, the avoidance of the trawl by shrimp and the sampling efficiency. Horizontal microstructures and the variability of the vertical distribution of shrimp in the vicinity of the bottom layer sampled by the trawl may also be density dependent. The relative importance of these factors that all contribute to the high nugget variability is, however, unknown. This question deserves more research efforts.

At small distances of about 15 km, the slopes of the variograms of each stratum changed markedly. This may indicate that the shrimp tended to aggregate in mesoscale patches, having a diameter which was twice this distance, but the sampling grid used was too coarse to confirm it through the structure function. The kriged maps showed, however, four rich areas of 700–2000 km^2. Increasing the local density of samples is therefore recommended to more precisely define the intercept of the spatial structure model and to verify the existence of this mesoscale pattern.

The chief macrostructures evidenced on the kriged maps was the rarity of the shrimp along the southern shelf of the Laurentian Channel and a tendency of being richer in the northwest. The reason for this is unclear, since the sediment type (Loring and Nota 1973) and bottom temperatures (2–6°C)

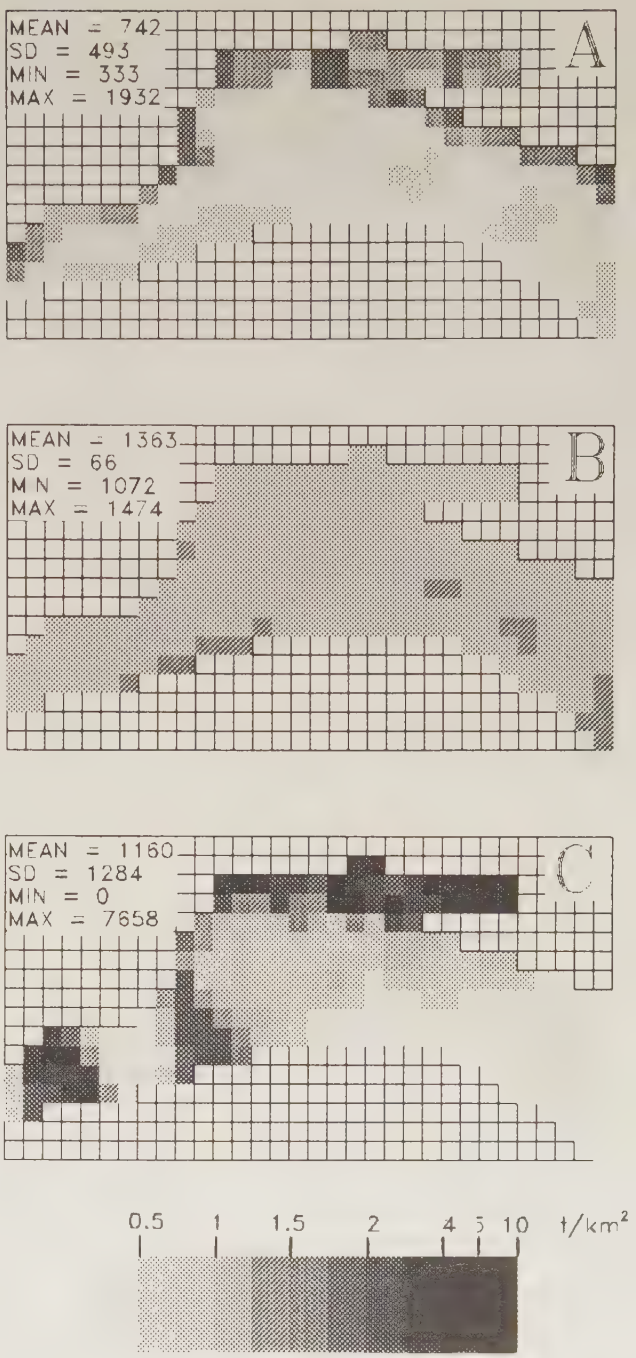

FIG. 6. Maps of the kriging SD's corresponding to the three kriging schemes presented in Table 1: (A) two-strata variograms; (B) whole region traditional variogram; (C) relative variogram. Point kriging σ_k^* ($\sigma_k^* \cdot Z^*$, in Fig. 6C) estimated at the nodes of the 10 × 10 km grid cells.

are favorable throughout the sampled region. This structure is the source of the trend we observed. It is an important regional feature of the shrimp spatial organization, which has a high degree of persistence, from unpublished shrimp fishery catch and research data. Many mechanisms can be invoked to generate and maintain this structure. We suggest the following circulation hypothesis. The location of the region at an elbow of the Laurentian Channel and the presence of a basin in the northeast surely affect the deepwater circulation and might favor the "retention" of the shrimp and/or their food in the rich areas

TABLE 3. Estimated mass of shrimp ($\times 10^3$ kg) in the study region for four cutoff densities according to discrete summation of the kriging punctual estimates (kg/km^2) at the nodes of the interpolation grid (equation (6)) or to numerical integration with the Simpson 3/8 rule, for the chosen kriging methods (Table 1), polygonal tessellation, and the arithmetic mean. The relative errors (SE = σ_E/total biomass) are computed from equation (11) for the kriging methods and from classical statistics for the polygonal tessellation.

	Discrete summation				Simpson integration	
Method and cutoff	Mean (kg/km^2) ×	area (km^2) =	mass (t)	SE (%)	Area (km^2)	Mass (t)
Cutoff: >0 kg/km^2						
Traditional variogram	933.00	22 800	21 272	8.7	23 241	21 684
Relative variogram	912.71	24 100	21 996	9.7	24 596	22 449
Two-strata variograms	911.28	24 100	21 962	6.5	24 650	22 463
Arithmetic mean						
Whole region	1249.28	24 300	30 358			
Two-strata	2391.73	6 500	15 546			
	571.77	17 800	10 178			
	1058.69	24 300	25 724			
Polygonal tessellation	818.68	23 214	19 005	13.1		
Cutoff: ≥500 kg/km^2						
Traditional variogram	1621.99	11 800	19 139	7.1	12 120	19 658
Relative variogram	1498.21	13 400	20 076		13 902	20 828
Two-strata variograms	1538.57	13 000	20 001		13 492	20 759
Cutoff: ≥1000 kg/km^2						
Traditional variogram	2454.76	6 100	14 974	5.9	6 292	15 444
Relative variogram	2198.44	7 100	15 609		7 463	16 404
Two-strata variograms	2279.43	6 900	15 728		7 247	16 518
Cutoff: ≥1500 kg/km^2						
Traditional variogram	2913.60	4 500	13 111	5.8	4 626	13 478
Relative variogram	2563.41	5 100	13 073		5 447	13 961
Two-strata variograms	2779.76	4 700	13 065		4 980	13 844

where they are found. The residual circulation in the region is upstream on the northern side of the channel and downstream on the southern side (Trites and Walton 1975). The cumulative effect of this cyclonic circulation on the shrimp, during their excursions in the water column at night (Simard et al. 1990), would result in a higher probability of (1) retention of the shrimp (and their food) upstream, north, and (2) exportation downstream, outside of the region on the southern side.

Kriging Performance

The variograms indicate the presence of a structure, but very little of this information was useful for the estimation, as indicated by the jackknife cross-validation tests. The best reestimations were always associated with a small number of information points, which contradicts the idea that taking the spatial structure into account is advantageous when computing the local estimates. This resulted from the two above-mentioned factors: the high unresolved variability and the lack of stationarity.

When stationarity does not hold and when the variance increases with the value of the variable, efforts must be made to minimize the deviation from stationarity, at least locally. This can be done in many ways such as removing the trend and kriging on the residuals, transforming the data to logarithmic units, computing relative variograms, stratifying the region in more homogeneous strata, or using more elaborate geostatistical models. All these tools have, however, their own limitations and conditions of application. The relative variogram appraoch used here gave no advantage for modeling the spatial structure, its results being worse than the traditional variogram for the whole region. Likewise, the logarithmic transformation was probably inappropriate for the type of nonstationarity encountered. The partitioning of the region into two more homogeneous strata provided a better fit of the spatial model. This approach gave a SD of the errors of the estimates of the spatial model over the whole region of 718 kg/km^2, which was 21% lower than the whole-region approach with the traditional variogram (SD_E = 914 kg/km^2). The importance of having the proper structure model in each stratum (or the whole region) to minimize the SD of the errors of the estimates was also clearly depicted in the cross-validation trials. The drawback of stratifying is that it requires more data handling and computation than with the whole-region approach, and the number of strata is limited by the number of points needed to compute a significant variogram (>50 points). This extra work was worthwhile because our ultimate goal is to identify *significant* changes in the temporal evolution of the stock, which is in part dependent on the narrowness of the confidence intervals of the estimates.

The three kriging models produced very similar global and cutoff estimates and maps of the shrimp biomass, but their estimated errors were quite different. As expected, the stratification of the region in two strata of different density and variability produced the smallest SE of the global estimation. The gain was a SE reduction by 25% compared with the whole-region traditional approach. That the errors of the cutoff estimates above increasing threshold densities of shrimp were not

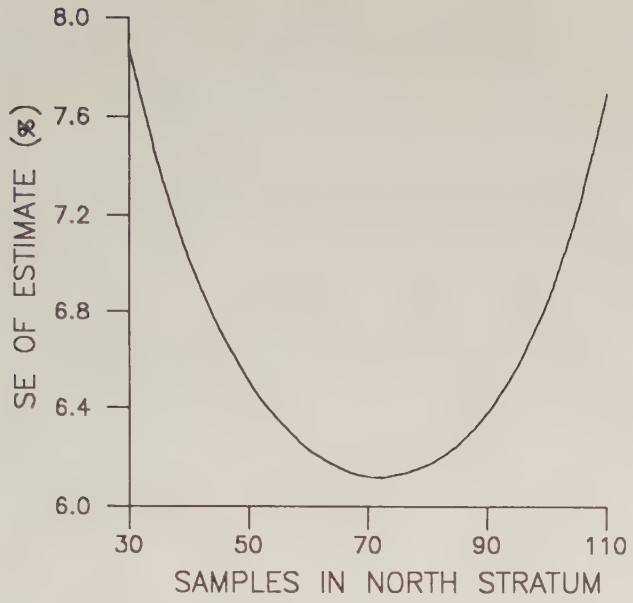

FIG. 7. Standard error of the global estimate (SE = σ_D/total biomass) as a function of the number of samples in the north stratum for the two-strata model for a fixed total number of 137 samples.

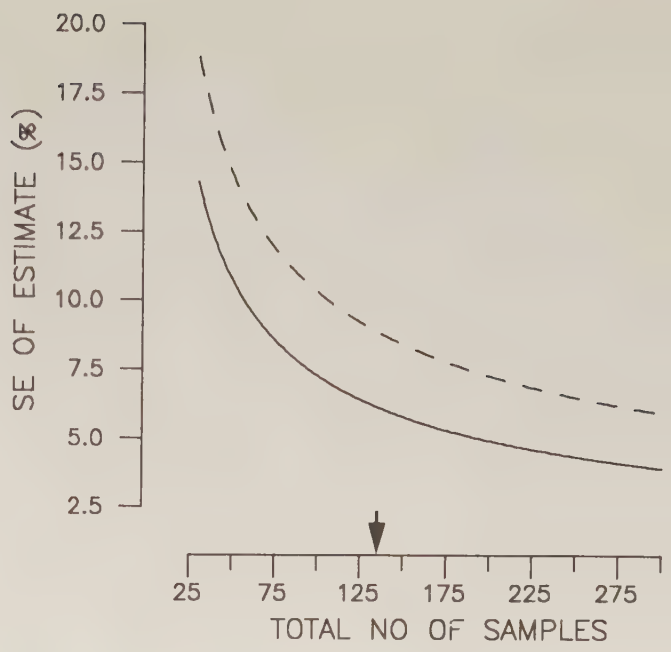

FIG. 8. Standard error of the global estimate (SE = σ_D/total biomass) as a function of the total number of samples for the two-strata geostatistical (solid line) and classical (broken line; σ^2 = variance of samples) models, for a fixed optimal allocation of 53% of samples in the north stratum from Fig. 7. The arrow indicates the present total number of samples.

larger than the error of the global estimate, but slightly smaller, indicates that the samples were adequately distributed relative to the density of shrimp.

These geostatistical errors of the global and cutoff estimates must be interpreted with caution. They are representing the error of the estimate according to the chosen structure model. If this model adequately describes the spatial structure, then the computed errors of the estimates are appropriate. The cross-validation of our optimal kriging models showed that they reproduced the true sample values with a correlation of about 0.70. This is the minimum correlation we may therefore expect from the kriging estimates because they were computed with closer information points (always smaller than the distance between samples) than the cross-validation estimates, where the information points were more distant (on average equal to distance between samples). An increase of the sample density would increase this correlation of the spatial model and reduce the errors of the global and cutoff estimates computed from equation (11) by reducing the surface of influence of the samples. At these small scales, the presence of a structural variability and its consideration in computing the errors of the estimates in equation (11) would result in a higher error reduction than it would in the random case, which is assumed in classical statistics. This advantage of taking into account the spatial structure was also clearly depicted here; kriging always produced much better estimations (with smaller errors) than the alternative polygonal method, which did not involve a structure function.

Sampling Optimization

Stationary geostatistics tells us that the variance of the estimates of a spatially structured variable will be minimized by sampling over a regular grid (Hughes and Lettenmaier 1981; Munoz-Prado et al. 1989). This can be seen from equations (9) and (10) where the size and geometry of the area of influence of the samples are involved in the computation of the variance of the global estimate. The precision of this estimate will therefore be partly determined by the spacing between samples. When geometric anisotropy is present, the grid should be stretched in the direction of the smallest variability by the ratio of the slopes of the directional variograms computed for the directions of minimum and maximum variances. This distribution of samples also ensures obtaining the optimal map of the variable over the whole studied region.

These theoretical guidelines are true only if the structure function is adequate and perfectly known. This is not the case in practice, since the variograms are unknown and they must be computed from the samples. Unless the grid is very tight, the definition of the structure function at small scales will not be precise enough to separate the structural from the random variability. It seems therefore worthwhile to direct a proportion of the samples to increase the local density of the grid (Warrick and Myers 1987; Fortin et al. 1989). This would enhance our knowledge of the small-scale variability and better define the variogram at small distance classes, in order to obtain a more precise estimate of the global variance. When the proportion of the structural variability at the scale corresponding to the separation between samples is small compared with the unresolved variability, the use of a regular grid instead of random sampling will not significantly improve the precision of the global estimate. It will, however, offer the advantage that the map will have a uniform level of uncertainty. Drawbacks of a regular grid are the risks of aliasing, when cyclic variations are interfering with the sampling frequency, and of having an improper sampling step relative to the variability of the samples.

Results showed that stratification minimized the error of the global estimate. Could we reduce again this error by changing the allocation of the 137 samples between the two strata? Using equation (11), the SE of the global estimate was computed for different allocations of the samples between the two strata (Fig. 7). The minimum SE is obtained when 72 samples (53%

of total) are placed in the north stratum and 65 in the south stratum. This changes the allocation of 21 samples in favor of the north stratum compared with the present case. The SE of the global estimate associated with this reallocation is 6.1%, which is a slight reduction compared with the present value of 6.5%.

The effect of increasing or reducing the total number of samples of this optimal allocation is depicted in Fig. 8. Under the classical hypothesis of random distribution (pure nugget variogram), the SE of the global estimate decreases as a function of $n^{-0.5}$. It can be shown that SE decreases as $n^{-0.75}$ for a linear variogram and, provided n is sufficiently large, as $n^{-1.0}$ for a Gaussian variogram. For mixed models, this decreasing function will depend on the relative importance of each variogram component. Here, because of the high proportion of the unresolved nugget variability, the SE of the global estimate decreases with a rate close to the classical case. It was, however, always significantly lower than the SE computed under the hypothesis of randomness. Doubling the sampling effort would result in a reduction, by one third, of the SE of the estimate, which would become 4.1%. Decreasing the number of samples by one half would give a SE of 9.0%, which is a relative increase of about 50%.

Sampling in areas of high uncertainty could be reinforced using the error maps computed. Figures 6A and 6B reflect the sampling density in the stratum or region considered and point out areas where the sampling could be increased to get a better local estimate considering the local variability, independent of the value of this estimate. As expected from equation (5), the stratified scheme resulted in lower errors in the south stratum in response to its lower variance. In general, both maps point out the same areas of high errors in this stratum. The error depicted in Fig. 6C takes into account not only the sample density and local variability but also the values of the local estimates. It thus indicates the areas where it is worth increasing the sample density for estimation of the global biomass because they are sufficiently rich and they have a high variance or, conversely, decreasing the sampling density because of a uniform low abundance. For example, more samples should be collected in the south, at the junction of the gulf and the estuary, to delineate more accurately the extent of the local rich patch (Fig. 5D and 6C). Similarly, the sampling effort in the southeast could be relaxed because of its low variance.

To conclude on the geostatistical estimation of the biomass of the northern shrimp in the Gulf of St. Lawrence, the nonstationarity and the high unresolved small-scale variability are two questions that need further research in order to more precisely define the spatial model. The use of more exhaustive data sets and data from repeated surveys would surely bring more information on the shape of the probabilistic model driving the spatial organization of the shrimp. Despite this limitation the present results show that geostatistics can easily provide various precise estimates for spatially autocorrelated biological resources. Besides, it provides extra information on their spatial organization, which can hardly be obtained otherwise, and which is most helpful for understanding the ecology of the studied species and, hence, for improving their management.

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Relation longueur/écaille allométrique chez le saumon atlantique (*Salmo salar*) durant la phase marine

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Nous avons précisé la relation longueur/écaille chez le saumon atlantique (*Salmo salar*) de la rivière St-Jean, près de Gaspé, Québec. Une analyse de covariance indique que la relation isométrique, généralement admise durant la phase marine chez cette espèce, est conservée si on n'utilise que des écailles d'adultes, mais pas si des écailles de saumonneaux sont incluses dans l'analyse. Ces résultats suggèrent une courbure de la relation longueur/écaille durant la première année de mer. Une équation semi-logarithmique ($Y = e^{2,15 + 0,57X}$; $P < 0,01$, $R^2 = 0,988$) permet de rétrocalculer correctement les tailles à la smoltification des saumons adultes de la rivière St-Jean. La différence de distribution des tailles à la smoltification observées et rétrocalculées suggère une mortalité sélective en fonction de la taille chez les saumonneaux dans les premiers temps de leur séjour en mer.

The body/scale relationship of Atlantic salmon (*Salmo salar*) was determined for the stock of the St. Jean River, near Gaspé, Québec. A covariance analysis shows that the isometric relationship generally admitted for the marine phase holds when we use adult scales only, but must be rejected when smolt scales are included in the analysis. These results suggest a curvature of the body/scale relationship during the first year at sea. A semi-logarithmic fit ($Y = e^{2.15 + 0.57X}$; $P < 0.01$, $R^2 = 0.988$) allows correct backcalculation of length at smolting for adult salmon of the St. Jean River. The difference between observed and backcalculated distribution of smolt length suggests a length-related selective mortality for smolts at the beginning of their marine life.

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Malgré que les méthodes de rétrocalcul à partir de mesures d'écailles aient été développées entre autres pour le saumon atlantique (*Salmo salar*), peu d'études détaillées existent sur la relation entre la longueur corporelle et le rayon de l'écaille (dorénavent notée relation longueur/écaille) chez ce salmonidé. L'étude la plus complète est due à Lindroth (1963) qui réfère à celles de Dahl (1910), Lindsay et Thompson (1932) et Kerr (1961).

Deux définitions sont requises pour aborder cette question. Lorsqu'il existe une proportion constante, durant toute la vie d'un organisme, entre deux parties ou une partie et la totalité de cet organisme, on dit qu'il y a isométrie. Si cette proportion n'est pas constante, on dit qu'il y a allométrie (Tessier 1948; Bagenal et Tesch 1978; Smith 1980; Reiss 1989). La relation linéaire sera qualifiée dans cet article d'isométrique, bien que sa place dans les définitions qui viennent d'être données ne soit pas claire.

Pour le rétrocalcul des longueurs chez les saumons adultes, on utilise généralement une relation de proportionnalité (Lindsay et Thompson 1932; Lindroth 1963; Anonyme 1984). Ce faisant, on pose une hypothèse d'isométrie. Cependant, il semble bien que chaque stade (tacon, saumonneau et adulte) soit caractérisé par une relation longueur/écaille qui lui soit propre (Kerr 1961; Lindroth 1963). Comme cette étude porte sur la relation longueur/écaille durant la période marine, celle-ci a été établie à partir d'un échantillon couvrant toute la gamme des tailles que l'on désire rétrocalculer (Carlander 1981). De cette façon, il sera possible ultérieurement de rétrocalculer les longueurs durant le séjour en mer, et de comparer les saumons uni, di et tribermarins (saumons respectivement d'une, deux et trois années en mer) sur la base de l'âge, des longueurs à l'avalaison et des taux de croissance. L'étude porte sur la relation longueur/écaille du stock de saumons atlantique de la rivière St-Jean, près de Gaspé, Québec.

Matériel et méthode

Les écailles utilisées pour cette étude ont été prélevées sur des saumonneaux et des adultes provenant de la rivière St-Jean (Gaspé), au Québec. Les saumonneaux sont capturés sur la barrière de comptage du Ministère du Loisir, de la Chasse et de la Pêche du Québec (MLCP). Les adultes sont pris soit au filet-trappe dans l'estuaire de mai à juillet par les employés du MLCP, soit par des pêcheurs sportifs.

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Des saumonneaux sont collectionnés au hazard sur la barrière de comptage, à chaque jour, pour caractériser le groupe en dévalaison (longueur totale et à la fourche, poids, sexe, âge par les écailles, etc.) (Ouellet et Turgeon 1987; G. Ouellet et M. Turgeon, données non-publiées). Dans le cas des adultes capturés au filet-trappe, les employés du MLCP mesurent la longueur à la fourche des spécimens et prélèvent quelques écailles-clés (Lindroth 1963). Dans le cas des saumons capturés sportivement, lors de l'enregistrement, les préposés mesurent la longueur à la fourche et prélèvent quelques écailles entre la fin de la nageoire dorsale du saumon et la ligne latérale. Il s'agit dans ce cas d'écailles standard. Il a été démontré (chez *Micropterus dolomieui*) que les relations longueur/écaille établies à partir de ces deux types d'écailles ne différaient pas statistiquement (Everhart 1950, *dans* Warner et Havey 1961).

Les écailles et les longueurs utilisées dans cette étude ont été extraites des collections du MLCP par échantillonnage à pas systématique (Frontier 1983). Dans le cas des saumonneaux, deux sous-échantillons ont été extraits de chacun des échantillons de 1982, 1984 et 1985 (celui de 1983 ne contenait que 23 individus). Le premier sous-échantillon ($N = 103$) a été prélevé pour établir la relation longueur/écaille. Le second sous-échantillon ($N = 546$), ne contenant que des longueurs, était destiné à une comparaison avec les tailles à la smoltification rétrocalculées chez les adultes. Comme l'échantillon de 1982 ne contenait que 182 individus, cette taille du sous-échantillon confère un poids statistique égal aux trois années en cause. Dans le cas des adultes, le sous-échantillon ($N = 338$), provenait des années 1983 à 1986, et contenait respectivement 98, 66, 102 et 72 écailles et mesures de longueurs.

Les écailles de saumonneaux ont été montées en frottis sur des lames de plastique collées à chaud. Les écailles d'adultes ont été nettoyées dans de l'eau savonneuse et examinées une première fois afin de sélectionner des écailles symétriques dont la bordure était intacte, sans marques de fraye et possédant le foyer original. Selon Anonyme (1984) celui-ci doit mesurer moins de 0,5 mm du centre au premier circulus. Des écailles avec un foyer au diamètre inférieur à 0,4 mm furent sélectionnées exclusivement car il a été démontré que dans les rivières d'eau très froide, telle la St-Jean, le premier annulus peut manquer (Jensen et Johnsen 1982). Les écailles sélectionnées ont ensuite été montées entre une lame de verre et une lamelle.

L'âge, en rivière et en mer, a été déterminé et inscrit sur chaque lame selon la notation usuelle dans le cas du saumon (Anonyme 1984). Une des écailles montées fut alors mesurée à l'aide d'un analyseur d'image (Optical Pattern Recognition System, Biosonics Inc.). La mesure de la radiale médio-antérieure a été effectuée pour chaque marque annuelle (annulus) à la limite extérieure de la zone hivernale.

Afin de vérifier si l'utilisation d'une seule écaille par individu pouvait induire une variabilité supplémentaire dans la relation longueur/écaille, des mesures portant sur quelques écailles provenant de 29 sujets adultes furent effectuées. Les résultats ont montré que la variance de l'écaille inter poisson est de loin supérieure à la variance intra poisson (analyse de variance : $dl = 28, 97$; $F = 17{,}01$; $p < 0{,}0001$).

Le rétrocalcul comporte deux étapes. Premièrement, la relation longueur/écaille est analysée statistiquement afin d'obtenir une équation décrivant, généralement, la longueur en fonction de l'écaille. Deuxièmement, les mesures des annuli sont converties en longueurs à l'aide des paramètres de l'équation obtenue lors de la première étape.

La première étape du rétrocalcul pose certains problèmes biostatistiques. En effet, la relation longueur/écaille n'étant pas forcément linéaire, employer une régression linéaire pour l'analyse ne permet pas une comparaison claire entre les ajustements réalisés sur les données transformées pour linéariser la relation (Bartlett *et al.* 1984).

Par ailleurs, ajuster une régression linéaire par la méthode des moindres carrés (régression prévisionnelle) suppose que la variable dépendante (dans ce cas la longueur) est responsable de toute la variabilité de la relation longueur/écaille, ce qui est biologiquement et statistiquement injustifiable. Tessier (1948) et Ricker (1973, 1984) ont suggéré l'utilisation de régressions fonctionnelles pour séparer la variance entre les deux variables.

Enfin, Smale et Taylor (1987) considèrent que l'estimation la plus précise des paramètres de l'équation décrivant la relation longueur/écaille est obtenue avec les moyennes des radiales par classes de longueurs. Cette procédure permet de réduire et stabiliser la variance due à l'écaille.

Afin d'étudier objectivement les relations longueur/écaille, Bartlett *et al.* (1984) suggèrent d'utiliser une analyse de covariance, ce qui permet de fonder le choix de l'équation du rétrocalcul sur une base systématique. Cette analyse, également décrite par Snedecor et Cochran (1980), a donc été appliquée au cas du saumon atlantique. Une régression linéaire est appliquée sur l'ensemble des données, ensuite sur chacun des groupes obtenus à partir de l'âge en mer des poissons à la capture, et finalement, sur la valeur moyenne des longueurs et des radiales d'écailles pour chaque groupe d'âge (les barycentres). La variance de la relation longueur/écaille est ainsi répartie selon ses sources d'origine. Chacune est soumise à un test dans l'ordre suivant : la différence entre les pentes des régressions sur les groupes indique si une transformation des données est requise; la déviation des barycentres par rapport à une droite renseigne sur la linéarité de la relation; la différence entre les pentes des régressions sur les groupes et la pente de la régression sur les barycentres établit la part de la variance due à la variabilité de l'écaille; et enfin, si aucune autre source n'est significative, la régression d'ensemble indique la force du lien entre la longueur et l'écaille. Comme la variance des régressions sert de base aux tests et qu'elle est la même pour une régression fonctionnelle que prévisionnelle (Ricker 1973), la méthode apparait tout à fait justifiée pour cette étude.

Afin de respecter les conditions d'application de l'analyse de covariance, la normalité des données de longueurs et de radiales a été vérifiée par un test de Kolgomorov–Smirnov. Un test d'égalité des variances (F_{max} de Hartley; Sokal et Rohlf 1981), suggérait d'effectuer une transformation des données avant de procéder à l'analyse lorsque les saumonneaux étaient inclus. Une transformation log–log fut donc effectuée dans ce cas.

Pour la seconde étape des rétrocalculs, Carlander (1981) et Gutreuter (1987) soulignent que la conversion la plus exacte des radiales d'écailles en longueurs est obtenue par la méthode proportionnelle. L'équation pour la conversion est :

$$(1) \qquad L_i = a + \frac{(L_c - a)}{R_t} R_i$$

où L_i est la longueur à un âge donné, L_c est la longueur à la capture, R_t est la radiale totale de l'écaille, R_i est la radiale à l'annulus considéré et a est l'ordonnée à l'origine de la régression linéaire ajustée. L'équation 1 est celle de Fraser–Lee. Lorsque le paramètre a est égal à zéro, l'équation pour le rétro-

TABLEAU 1. Analyse de covariance de la relation longueur/écaille chez a) les adultes de la rivière St-Jean pour les années 1983 à 1986 et b) les adultes et les saumonneaux pour les années 1982 à 1986.

Source de la variation	Somme des carrés	dl	Carré moyen	F	p
		a) Adultes seuls, 1983 à 1986			
Total	28 467,78	338	84,22	—	—
Régression d'ensemble	5 591,26	1	5 591,26	406,90	<0,001
Différences des groupes et des barycentres	18 271,12	1	18 271,12	1 329,67	<0,001
Déviation des barycentres de la ligne droite	12,57	1	12,57	0,91	>0,50
Entre les pentes des groupes	17,03	2	8,51	0,61	>0,50
Résiduelle	4 575,78	333	13,74	—	—
		b) Adultes et saumonneaux, 1982 à 1986			
Total	46,207	440	0,105	—	—
Régression d'ensemble	44,566	1	44,566	75 434,83	<0,001
Différences des groupes et des barycentres	1,262	1	1,262	2 136,74	<0,001
Déviation des barycentres de la ligne droite	0,089	2	0,044	76,03	<0,001
Entre les pentes des groupes	0,032	3	0,010	18,29	<0,001
Résiduelle	0,256	434	0,0005	—	—

calcul est celle de Dahl–Lea (Hile 1970; Bagenal et Tesch 1978; Bartlett *et al.* 1984).

Résultats

Afin de tester l'hypothèse d'une relation longueur/écaille isométrique généralement posée, une analyse de covariance a été effectuée sur le groupe des adultes seulement (données non-transformées). Les résultats indiquent que l'hypothèse d'isométrie est tout à fait justifiée (tableau 1a; $F = 0,91$). La régression sur les barycentres des groupe d'âge est effectivement linéaire ($p = 0,014$, $R^2 = 0,999$).

Lorsque les données relatives aux adultes et aux saumonneaux sont utilisées, après transformation log–log pour rendre les variances homogènes (ce qui revient à tester la relation $Y = aX^b$, dite d'allométrie), l'hypothèse d'isométrie doit être rejetée (tableau 1b, $F = 76,03$). L'hypothèse d'isométrie est remise en question entre le stade saumonneau et le stade adulte. Il faut admettre une courbure de la relation longueur/écaille durant la première année de mer si l'on veut rendre compte de la pente de la relation aux stades saumonneau et adulte (fig. 1).

Il devient donc nécessaire d'ajuster une courbe sur l'ensemble adultes-saumonneaux. Kerr (1961) avait suggéré l'utilisation d'une forme quadratique pour l'ensemble adultes-tacons, mais les unibermarins se retrouvent surtout à droite de la courbe et les tribermarins à gauche selon cette méthode (fig. 1). Le même phénomène se produit d'ailleurs si l'on utilise une droite pour rétrocalculer les longueurs au stade saumonneau. La situation est semblable si l'on utilise l'équation d'allométrie généralement proposée pour des cas similaires (Tessier 1948; Sokal et Rohlf 1981; Conan et Comeau 1986; Reiss 1989).

Par ailleurs, les résultats indiquent également que la variabilité de l'écaille influence la régression d'ensemble sur les adultes seuls (tableau 1a; $F = 1\,329,67$). La variabilité de l'écaille influence aussi la régression d'ensemble lorsque les saumonneaux sont inclus dans l'analyse, après la transformation log–log (tableau 1b, $F = 2\,136,74$). Il est donc nécessaire à ce stade de ne travailler qu'avec les radiales moyennes par classes de taille, comme le suggèrent Bagenal et Tesch (1978) et Smale et Taylor (1987), afin de réduire et stabiliser la

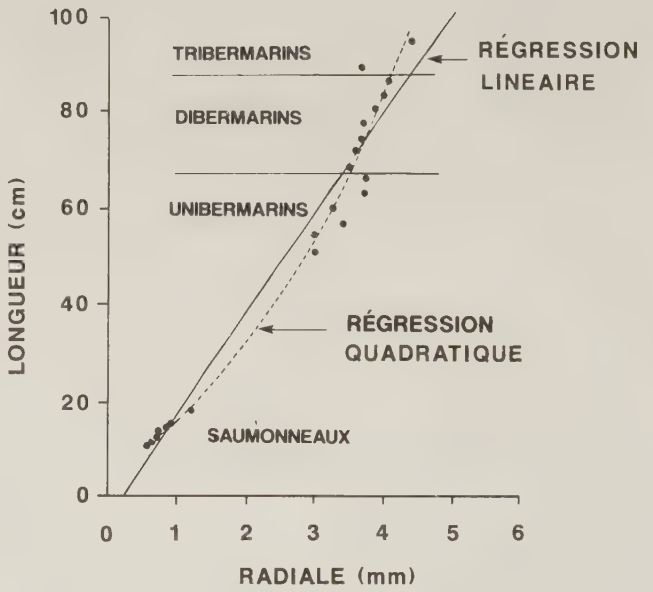

FIG. 1. Ajustement comparé d'une régression linéaire et d'une régression quadratique sur les classes de tailles en fonction des radiales moyennes d'écailles des saumons de la rivière St-Jean pour les années 1983 à 1986 ($Y = -3,74 + 20,72X$ pour la forme linéaire, $Y = 9,13 + 2,31X + 1,72X^2$ pour la forme quadratique; intervalles de 3 cm pour les adultes, 0,5 cm pour les saumonneaux).

variance due à l'écaille. L'analyse de covariance devient cependant inutilisable.

Le meilleur ajustement est obtenu en utilisant une relation semi-logarithmique sur les radiales moyennes par classes de taille (tableau 2). L'équation décrivant le mieux la relation longueur/écaille est donc :

$$(2) \qquad Y = e^{2,15 + 0,57X}$$

($p < 0,01$, $R^2 = 0,988$). Cette équation respecte les conclusions tirées de l'analyse de covariance sur l'ensemble adultes-saumonneaux. Elle présente une pente similaire à celle des saumonneaux pour cette gamme de taille, et s'ajuste très bien

TABLEAU 2. Ajustement comparé de régressions de forme linéaire quadratique, allométrique et semi-logarithmique sur les classes de taille en fonction des radiales moyenne des saumons de la rivière St-Jean pour les années 1983 à 1986.

Modèle	dl	Carré moyen	F	R^2	Estimation des paramètres : a	b	c	Ecart à la moyenne : a	b	c
Linéaire	1	17 926,88	431,33	0,955	−3,74	20,72	—	3,06	0,99	—
Résidus	20	41,56								
Quadratique	3	26 813,18	905,60	0,970	9,13	2,31	1,72	4,85	5,94	4,95
Résidus	19	29,60								
Allométrique	1	13,62	1 311,57	0,985	2,88	1,06	—	0,03	0,02	—
Résidus	20	0,01								
Semi-log	1	13,66	1 654,41	0,988	2,15	0,57	—	0,04	0,01	—
Résidus	20	0,008								

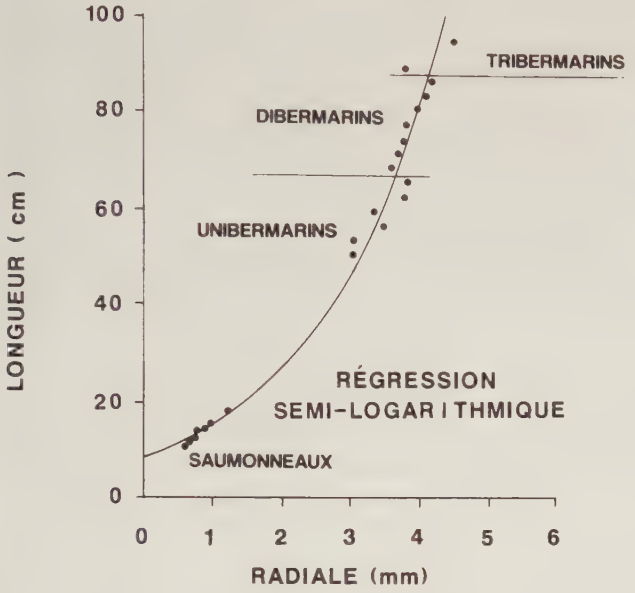

FIG. 2. Ajustement d'une régression curviligne de forme $Y = e^{2,05 + 0,57X}$ sur les classes de tailles en fonction des radiales moyennes d'écailles des saumons de la rivière St-Jean pour les années 1982 à 1986 (intervalles de 3 cm pour les adultes, 0,5 cm pour les saumonneaux).

aux moyennes des longueurs et des radiales, pour tous les groupes d'âge. De plus, elle laisse autant d'unibermarins (ou de tribermarins) de part et d'autre de la courbe (fig. 2).

Elle permet de rétrocalculer de façon satisfaisante les tailles à l'avalaison, en ramenant l'équation 2 sous une forme linéaire et en utilisant la méthode proportionnelle. On considère alors que chaque individu suit une relation longueur/écaille qui lui est propre et qui s'écarte graduellement de celle de l'ensemble de la population au fur et à mesure de la croissance. Cette hypothèse a déjà été suggérée par Bartlett *et al.* (1984) et Hile (1970). Ainsi, la déviation hypothétique entre l'écaille de l'individu et l'écaille moyenne de la population, dans la relation $\ln Y = a + bX$ (transformation logarithmique de l'équation 2), est proportionnelle au logarithme de la longueur. Sous la forme de l'équation 2, l'individu qui s'écarte de la moyenne de la population suit une relation curviligne similaire à la relation longueur/écaille de la population. Les tailles rétrocalculées ont été corrigées pour l'emploi d'une équation logarithmique (Sprugel 1983).

On a comparé les tailles observées des saumonneaux sur la barrière de comptage à celles rétrocalculées pour les adultes au même stade (tableau 3). Les tailles rétrocalculées à partir d'écailles d'adultes présentent une moyenne de taille semblable à celle observée des saumonneaux sur la barrière de comptage du MLCP ($t = 1,822$; $p = 0,07$). La distribution est différente (test de Kolmogorov–Smirnov, $dN = 0.174$, $p < 0,0001$). Il y a en proportion plus d'individus de petite et de grande taille à l'avalaison chez ceux rétrocalculés que chez ceux observés (fig. 3). Cette distribution peut être interprétée de deux façons. Elle peut suggérer, si on attribue plus d'importance à la moyenne pour comparer les distributions, que les individus de taille intermédiaire subissent une plus grande mortalité durant la période marine. Ou encore, si on prend en considération le mode, que la distribution rétrocalculée subit un aplatissement dû à des facteurs autres, statistiques ou biologiques, non-considérés durant l'analyse. Le plus grand étalement vers la droite suggérerait alors une meilleure survie des plus grand individus à l'avalaison.

TABLEAU 3. Comparaison entre les tailles (cm) observées des saumonneaux à la barrière de comptage du MLCP sur la rivière St-Jean pour les années 1982, 1984 et 1985 et celles rétrocalculées à partir des écailles d'adultes de 1983 à 1986.

Statistique	Calculé	Observé
Nombre de cas	338	546
Moyenne	12,31	12,48
Mode	11,25	11,75
Variance	2,73	1,42
Minimum	9,43	9,60
Maximum	19,78	17,70
Biais (skewness)	0,82	0,36

Discussion

La comparaison des résultats de l'analyse de covariance sur des données avec ou sans saumonneaux met clairement en évidence l'existence d'une allométrie de la relation longueur/écaille durant la période marine chez le saumon atlantique. En effet, l'inclusion des saumonneaux diminue la rectilinéarité de la relation ajustée sur les moyennes de longueurs et de radiales par groupe d'âge. Cependant, l'hypothèse d'isométrie généralement posée pour les adultes apparait toujours pleinement justifiable. Par conséquent, la nature des résultats nous amène à admettre une courbure durant la première année de mer (entre les stades saumonneau et unibermarin) qui ne peut être mise en évidence que par l'inclusion d'écailles de saumonneaux.

Par ailleurs, la pente de la droite ajustée sur les barycentres des groupes d'âge diffère significativement des pentes des

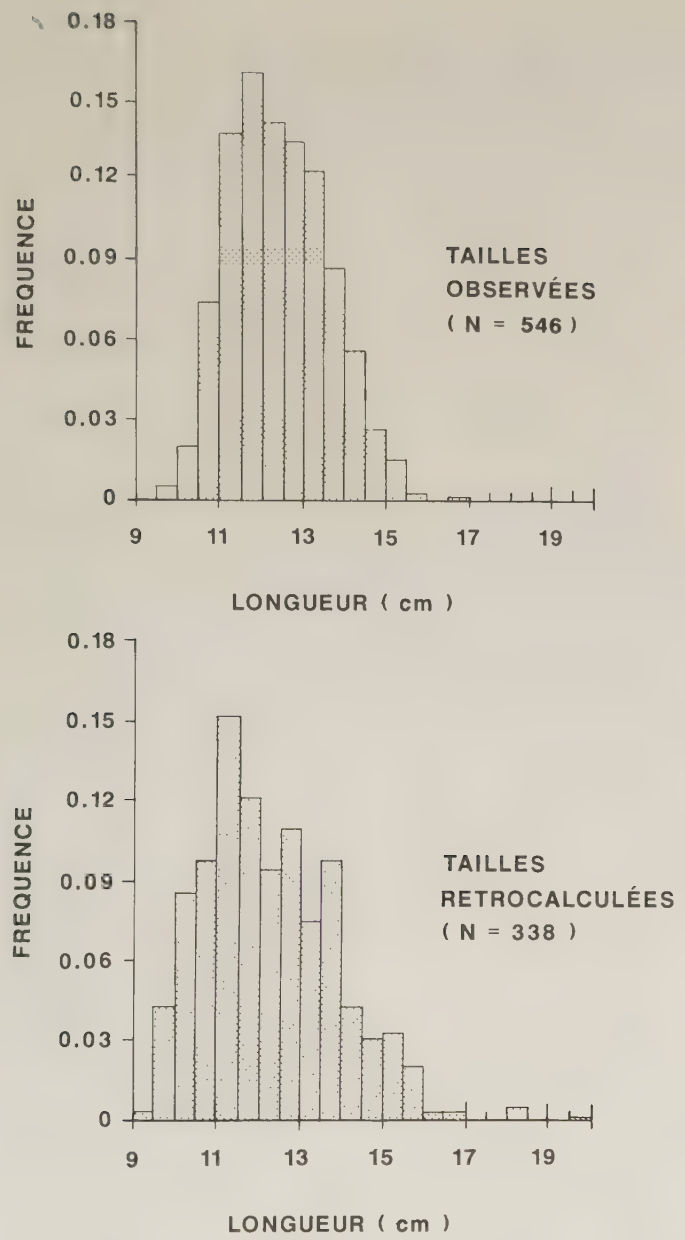

FIG. 3. Histogrammes de fréquence relative comparant les tailles d'avalaison rétrocalculées à partir d'écailles d'adultes et celles observées chez des saumonneaux en dévalaison sur la rivière St-Jean (tailles observées : 1982, 1984 et 1985; tailles rétrocalculées : 1983, 1984, 1985 et 1986).

droites ajustées sur chaque groupe d'âge (la covariable utilisée ici étant l'âge en mer des saumons). La variabilité de l'écaille influence donc les rétrocalculs basés sur une régression ajustée sur l'ensemble. Le rétrocalcul des tailles à l'avalaison à partir d'une régression linéaire sur des mesures d'adultes et de saumonneaux produit d'ailleurs des valeurs trop faibles ou trop fortes.

L'équation d'allométrie généralement utilisée dans de telles situations (Tessier 1948; Smith 1980; Sokal et Rohlf 1981; Conan et Comeau 1986; Reiss 1989) permet d'égaliser les variances par la transformation log–log qu'elle suppose, mais ne donne pas de meilleurs résultats. En effet, l'exposant b dans l'équation d'allométrie $Y = aX^b$ (testée dans le tableau 1b) égalait 1,02, soit donc une équation très peu différente de la droite ajustée sur les données non-transformées d'adultes et de saumonneaux. Ce résultat s'explique par le fait que la relation d'allométrie ne vise pas à l'étude des tendances entre les groupes d'âge (Reiss 1989), ne permet pas d'identifier le problème lié à la variabilité de l'écaille et passe obligatoirement par l'origine.

Le meilleur ajustement est obtenu à l'aide d'une régression semi-logarithmique sur les classes de taille en fonction des radiales moyennes d'écailles (tableau 2). La forme semi-logarithmique respecte les moyennes de longueurs et de radiales par groupe d'âge (fig. 2) et permet de rétrocalculer adéquatement les tailles à l'avalaison (tableau 3).

Cependant, il est clair qu'une telle forme, qui permet de calculer la taille des saumonneaux, va surestimer systématiquement la taille des tacons. Il faut donc admettre une courbure supplémentaire de la relation longueur/écaille entre les stades tacon et saumonneau, ce qui avait déjà été souligné par Kerr (1961) et Lindroth (1963).

La distribution des tailles à l'avalaison rétrocalculées suggère que les saumonneaux de taille intermédiaire subissent une plus grande mortalité durant leur séjour en mer (fig. 3), ou encore, que l'aspect plus étalé vers la droite de la distribution, déjà présent au stade saumonneau, est accentué par une meilleure survie des plus grands saumonneaux. Il existe des cas documentés de mortalités sélectives chez les saumonneaux. Ainsi il a été démontré, mais pas nécessairement admis, que la survie pouvait être corrélée positivement avec la taille des saumonneaux (Chadwick 1987; McCormick et Naiman 1984). Ritter (1989) rapporte par ailleurs que les individus migrant au début et à la fin de la période de migration vers la mer survivaient moins bien.

L'utilisation d'une relation curviligne suggère que la croissance des saumons lors de leur première année de mer est surestimée par les équations linéaire et quadratique. A cause du biais systématique introduit par ces formes (les unibermarins à droite de la courbe, les tribermarins à gauche), la croissance des saumons d'un an de mer est sous-estimée par rapport à celle des autres adultes durant la première année de mer, et celle des saumons de trois ans surestimée. Cette constatation vient éclairer sous un angle nouveau les différences significatives notées par Lear et Misra (1978) entre les écailles de saumons d'un et de deux ans de mer pour certains stocks des Maritimes, dans le nombre de circuli de l'annulus représentant la première année de vie en mer. On pourrait poser l'hypothèse que la croissance soit différente entre ces deux groupes d'âge en mer.

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Responses of the Freshwater Amphipod *Hyalella azteca* to Environmental Acidification

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Grapentine, L. C., and D. M. Rosenberg. 1992. Responses of the freshwater amphipod *Hyalella azteca* to environmental acidification. Can. J. Fish. Aquat. Sci. 49: 52–64.

Effects of acidification on the freshwater amphipod *Hyalella azteca* were investigated by examining the role of pH on natural distribution of the species in 30 northwestern Ontario lakes and by determining its responses to acidification in a series of field and laboratory experiments. The amphipod was absent from all five lakes that had pH 5.03–5.40 or Ca concentration <1.24 mg/L and was present in 23 of the 25 remaining lakes that had pH > 6.0 and Ca > 1.24 mg/L. In the field and laboratory experiments, amphipods transplanted to acidified lakes (pH 5.45, 5.66) or held in 500-L bags (pH ≤ 5.8) or 4-L aquaria (pH ≤ 5.6) showed lower survival than those held in corresponding neutral-pH, control treatments ($P < 0.05$). Neonate *H. azteca* were less tolerant of low pH than those in later life-history stages ($P < 0.05$). Populations of *H. azteca* will decline in habitats where the pH remains <5.8, a condition extant in a substantial proportion of lakes in eastern North America.

Les effets de l'acidification sur l'amphipode d'eau douce *Hyalella azteca* ont été étudiés par examen du rôle du pH sur la répartition naturelle de l'espèce dans 30 lacs du nord-ouest de l'Ontario et évaluation des réponses à l'acidification par essais sur le terrain et en laboratoire. Les amphipodes étaient absents des cinq lacs où le pH se situait entre 5,03 et 5,40 ou dont la concentration en Ca était inférieure à 1,24 mg/L. Les amphipodes étaient présents dans 23 des 25 autres lacs dont le pH était supérieur à 6,0 et la concentration de Ca supérieure à 1,24 mg/L. Au cours des essais sur le terrain et en laboratoire, les amphipodes placés dans des lacs acidifiés (pH de 5,45 et 5,66) ou conservés dans des sacs de 500 L (pH ≤ 5,8) ou des aquariums de 4 L (pH ≤ 5,6) ont présenté un taux de survi inférieur à ceux des témoins en milieu de pH neutre ($P < 0,05$). Les individus néonates étaient moins tolérants au faibles pH que ceux d'un stade de développement plus avancé ($P < 0,05$). Les populations de *H. azteca* diminueront dans les habitats où le pH demeurera inférieur à 5,8, un état qui prévaut actuellement dans une proportion appréciable des lacs de l'est de l'Amérique du Nord.

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Disappearance of benthic crustaceans is among the earliest and most marked responses of freshwater biota to acidification (e.g. Brehm and Meijering 1982; Nero and Schindler 1983; Raddum and Fjellheim 1984; Davies 1989). Loss of such sensitive organisms can disrupt food-web interactions and affect other organisms at pH's higher than those directly toxic to them (Schindler et al. 1985).

The freshwater amphipod *Hyalella azteca* is common and widely distributed in North America (Bousfield 1958; Pennak 1978), and yet only recently has it received attention in studies of the ecological effects of acidification (Stephenson and Mackie 1986; France and Stokes 1987). *Hyalella azteca* can dominate the zoobenthic community of the littoral zone of lakes, and thus is an important component of the food web (Weinert 1950; Cooper 1965; Bousfield 1970; de March 1981). Moreover, its widespread distribution and wide tolerance to most environmental conditions make its absence from a lake a potential indicator of an acidification problem.

The objectives of this study were to measure responses of natural populations of *H. azteca* to acidification and to estimate the lowest pH that would allow their long-term survival in a lake. The objectives were addressed by assessing factors regulating the natural distribution of *H. azteca* in a set of lakes in northwestern Ontario and by examining survival of the species under various experimental conditions in the field and laboratory.

Methods

Study Area

The Experimental Lakes Area (ELA) (93°30′–94°00′W, 49°30′–49°45′N) is located in northwestern Ontario, on the southwestern part of the Canadian Precambrian Shield (Cleugh and Hauser 1971). Thirty lakes in the area were included in the present study, and these had surface areas of 0.5–1007 ha (median = 12.2 ha) and maximum depths of 1 to >117 m.

ELA lake waters are very dilute and their chemical compositions are similar (table 1 in Grapentine 1987). Average concentrations of major cations are approximately 2.0, 0.9, 0.6, and 0.3 mg/L for Ca, Na, Mg, and K, respectively (Beamish et al. 1976). Average pH is about 6.5, and alkalinity is 40–80 μeq/L for most lakes (Schindler and Ruszczynski 1983). Nutrient and most trace metal concentrations are also low compared with lakes in other regions of the world (Prokopowich 1979). ELA lakes are low in productivity (Schindler and Holmgren 1971; Stockner 1971), and macrophytes are scarce in the littoral zones (Brunskill and Schindler 1971). The profundal zones of larger and deeper lakes are dominated by

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the amphipod *Pontoporeia affinis* and the mollusc *Pisidium conventus* whereas smaller lakes (mean depth <10 m) are dominated by chironomids and chaoborids (Hamilton 1971).

Several lakes at ELA have been used for whole-lake experiments. Acidification experiments have involved Lakes (L) 114 (1979–87), 223 (1976 to present), and 302 (1982 to present). Currently, L223 and L302S receive H_2SO_4 (Schindler 1985).

Distribution of *H. azteca* at ELA

Preliminary studies

In 1984, a preliminary survey of ELA lakes suggested that pH was affecting the distribution of *H. azteca*. Of 15 lakes sampled, only L223 and L302S, which were both experimentally acidified, did not have *H. azteca*. L223 and L302S had mean epilimnion pH's during the ice-free season of 5.4 and 5.6, respectively, whereas pH's of the other 13 lakes were >6.0. *Hyalella azteca* was present in L302S in 1983 (L. C. Grapentine, unpubl. data), at a mean pH of 5.9, so its disappearance the following year provided further evidence of a negative effect of low pH. Therefore, in 1985, a broader survey was undertaken to determine more precisely the effects of pH on the distribution of natural populations of *H. azteca*.

Selection of lakes for 1985 survey

Ca, Mg, temperature, and oxygen are known to affect the distribution of freshwater amphipods (Sutcliffe and Carrick 1973; Dadswell 1974; Schrimpff and Foeckler 1985; Økland and Økland 1986). Temperature and oxygen conditions in the littoral zones of ELA lakes were usually satisfactory for *H. azteca*, but the low concentrations of Ca and Mg were possibly limiting (de March 1981). Therefore, the lakes chosen represented a wide range of pH's and Ca and Mg concentrations. Water chemistry data from previous ELA surveys (Beamish et al. 1976; Schindler and Ruszczynski 1983) were used to select an initial group of lakes having pH's <6.0, alkalinities (<30 μeq/L, and Ca concentrations <1.5 or >3.0 mg/L (Mg concentration was correlated with Ca concentration). Another group of lakes having pH's >6.0, or Ca concentrations from 1.5 to 3.0 mg/L, was subsequently added, resulting in a total of 30 in the survey.

Water chemistry

Lakes were sampled in August and September 1985, a period of late summer stratification for ELA lakes (Schindler and Ruszczynski 1983). Duplicate water samples were collected from the littoral zone of each lake for measurement of pH and Ca and Mg concentrations. pH was measured with a Radiometer PHM4 pH meter and either an Orion Research 910200 electrode or Radiometer GK2401C combined electrode, calibrated with pH 4.00 and 7.00 buffers. Precision was at least ±0.05 pH unit. Samples for Ca and Mg analyses were filtered through Whatman GF/C filters, acidified, and concentrations determined by atomic absorption (Stainton et al. 1977). Details can be found in Grapentine (1987).

Collections of H. azteca

Preliminary collections of *H. azteca* in ELA lakes showed that highest densities occurred in substrates rich in allochthonous debris (leaves, twigs, bark), macrophytes, and emergent vegetation at depths <1 m, so sampling was confined to such habitats. A triangular dipnet was scraped once over suitable substrate in five different locations along the lakeshore, and the contents of each sweep were examined for amphipods. If no *H. azteca* were collected initially, an additional five areas were sampled. Up to 30 min was spent sampling for amphipods at each lake. Specimens, if present, were usually found within the first 5 min of searching.

Relative abundance of *H. azteca* in a lake was ranked using three categories: (a) "absent" if no individuals were found in 10 of 10 net sweeps, (b) "low" if individuals were found in ≤3 of 5 sweeps, or if individuals were found in 4 of 5 sweeps but averaged <10 per sweep, and (c) "high" if 5 of 5 sweeps contained individuals, or if individuals were present in 4 of 5 sweeps but averaged >10 individuals per sweep.

Field Experiments

Transplantation experiment

Transplantations were carried out in August 1984 to determine whether absence from acidified Lakes 223 and 302S was due to low pH. Groups of amphipods collected from unacidified L239 were placed in small enclosures and transferred into (1) lakes acidified with H_2SO_4 (L223 (mean epilimnetic pH 5.45) and L302S (pH 5.66)), (2) a lake acidified with HNO_3 (L302N (pH 6.32)), (3) a lake with neutral pH (L382 (pH 6.92)), and (4) back into L239 (pH 7.07) to assess the effects of containment (Fig. 1A).

Water temperature was measured at each site during each counting, and duplicate water samples were collected three or four times during the experiment for measurement of pH (Radiometer pH meter 29 and Fisher combination polymer body-gel filled electrode, or an Orion Research model 801 pH meter with 910200 electrode). pH's among transplantation sites within lakes and of sites through time were within ±0.1 unit (95% C.I.) of the lake mean in all lakes. Water temperatures were >20°C during the first 20 d of the experiment (10–27°C overall), and differences among lakes were ≤2°C on any given day.

Within each lake, enclosures were placed on the substrate at three widely separated sites at ≈1 m depth. Each enclosure contained 15 amphipods with similar proportions of large, medium, and small individuals. The enclosures, made of clear acrylic tube (7.6 cm inside diameter, 7.6 cm long) and 200-μm mesh screen, contained ≈2 g of dried allochthonous organic matter that provided food and cover and ≈0.25 mL of commercial fish food and alfalfa powder to supplement the food supply. Each enclosure was emptied periodically over 36 d and the living amphipods, including any offspring, were counted and then returned to the enclosures in the lake.

Group survival (i.e. the number or proportion of individuals surviving) after 19 d was compared among sites within lakes, and among lakes by ANOVA and multiple comparison of means using Fisher's protected least significant difference (LSD) test (Steel and Torrie 1980). The period of time chosen for analysis in this and the other field experiments depended on observations that the major effects had occurred.

Bag acidification

The bag acidification experiment examined survival in lake water of pH 5.6–6.3, a range hypothesized to include the lower tolerance limit of *H. azteca*. Single amphipods were placed into modified 22-mL polyethylene vials with screened windows and were held in eight bags of acidified lake water (500–550 L) placed in L239 (details in Grapentine 1987).

The pH of six of the eight bags was adjusted with dilute solutions of HCl and NaOH to reach intended H^+ concentrations of 1.00, 2.00, and 3.00 μmol/L. Actual mean concentra-

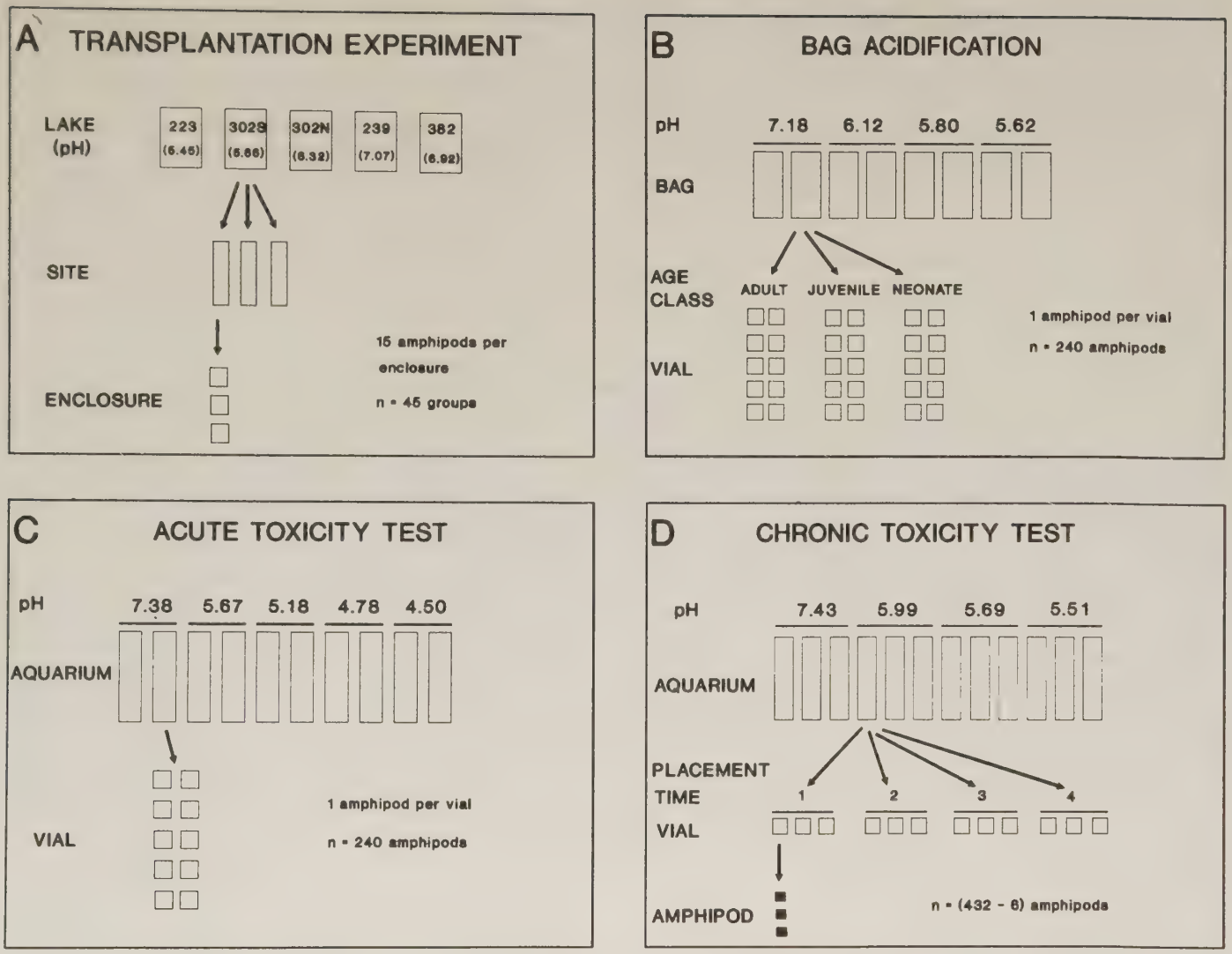

FIG. 1. Design of acidification experiments. (A) Transplantation experiment. pH shown for each lake is the mean of epilimnion samples taken from all three sites during the experiment. (B) Bag acidification. pH's shown are means of all measurements made from both replicates. (C) Acute toxicity test. pH's shown are means of all measurements made from both replicates. (D) Chronic toxicity test. pH's shown are means of all measurements made from the three replicates.

tions of duplicate acidified treatments were 0.76, 1.58, and 2.40 μmol H^+/L (=mean pH's of 6.12, 5.80, and 5.62) (Fig. 1B). Time-weighted mean pH's of replicates varied ±0.06 pH unit (95% C.I.). The pH of control replicates gradually increased from 7.00 to ≈7.25 during the experiment, resulting in a treatment mean of 7.18 (=0.066 μmol H^+/L). pH was measured every 1–2 d using equipment described above. Water temperature in the bags was measured periodically, and ranged from 14 to 20°C.

Amphipods for the experiment were collected from L239 (22 August 1985) and were separated into three size classes (adults: total body length ≥5 mm; juveniles: length ≈3 mm; and neonates: length ≤1 mm) to assess the effect of size on the ability of an individual to survive acidic conditions. Ten amphipods in each of three size classes were used (Fig. 1B), but they were not all placed into treatment at the same time. Vials housing amphipods contained a few plastic fibres for substrate and several flakes of commercial fish food (replaced as consumed). The 30 vials in each treatment were suspended in an acrylic holder just below the water surface (Grapentine 1987). Each amphipod was examined at ≈3-d intervals and its time of death (no movement when disturbed) was recorded. The experiment was terminated after 26 d.

Survival (and 95% C.I.) of all size classes within the bags was estimated by the product–limit (P-L) method described by Lee (1980). This method requires no assumptions about the statistical distribution of survival times, is suitable for small sample sizes, allows unequal times of placement of individuals into treatments,and can accommodate censored data (i.e. observations of individuals not followed until death), all conditions applicable to this experiment. Group survival calculated in this fashion was designated as a "survival function" ($\hat{S}(t)$) to distinguish it from the methods used to calculate survival in the other experiments (see transplantation experiment, above). The estimates of survival ($\hat{S}(t)$) for day 26 were compared by ANOVA and regressed against mean concentration of H^+ in bags.

P-L survival also was estimated for each size class within bags. Differences in survival between size classes within treatments were tested using a *K*-sample generalized Kruskal–Wallis *H*-test (Lee 1980). This nonparametric test is similar to a group of Wilcoxon-type tests, which are appropriate for sam-

ples with censored survival data (Lee 1980; Pyke and Thompson 1986), but the Kruskal–Wallis *H*-test allows use of more than two samples.

Laboratory Experiments

Acute toxicity test

This test examined acute lethality of low pH to *H. azteca* over a 5-d period. Laboratory-cultured individuals from the LaSalle River, Manitoba (49°45′N, 97°10′W), were held in 22-mL vials placed in duplicate 4-L glass aquaria of L239 water (Fig. 1C). A separate experiment to compare responses of LaSalle River and ELA populations failed because of high mortalities in the ELA controls. Mean alkalinity and Ca concentration of the test water were 151 μeq/L and 3.17 mg/L, respectively (G. Linsey, Department of Fisheries and Oceans, Winnipeg, Man., unpubl. data). pH was adjusted with weak solutions of HCl and NaOH to reach intended H^+ concentrations of 2.50, 7.50, 17.5, and 32.5 μmol/L. Actual mean concentrations were 2.14, 6.61, 16.6, and 31.6 μmol H^+/L (= pH 5.67, 5.18, 4.78, and 4.50) (Fig. 1C). Mean pH's of aquaria varied ±0.06 pH unit (average 95% C.I.). pH was measured two to four times daily, as described above. The unacidified control treatment had pH = 7.38.

Each aquarium was aerated. Water temperature was kept at 23°C and daylength was 16 h with fluorescent light producing irradiance of 100–200 μE/(m^2s), under which normal growth and reproduction should occur (de March 1981).

Amphipods collected from the culture were randomly placed into treatments on 13 May 1986. They were examined after 3, 9, and 18 h, and thereafter every 12 h for 5 d, and recorded as live or dead. All amphipods were preserved in 70% ethanol either after death or at the end of the experiment. The number of antennal segments (one of each of the first and second pairs of antennae) and head length (straight-line distance from the rostrum to the dorsal–posterior margin) were used to determine age of all specimens (Cooper 1965; Strong 1972).

Survival was analyzed in a number of ways. ANOVA was done for survival at 96 h, and mean survival in treatments was compared by Fisher's protected LSD procedure. Survival at 96 h was regressed against mean concentration of H^+ and mean pH of aquaria. To estimate 96-h LC50, survival data were transformed into logits, where logit $P = \ln(P/[1 - P])$, and P = proportion of individuals dead at time of observation (Ashton 1972), and regressed against time to obtain mortality rates in each aquarium (de March 1979). From these regressions, the expected time at which 50% of the amphipods would die was estimated for each aquarium, transformed to logarithms, and regressed against aquarium pH.

The effect of size or age on survival time should be more easily detected among 80 individuals than among 10. Therefore, individual survival times of amphipods within each aquarium were divided by the mean survival time of the 10 amphipods in that aquarium. The resultant "relative survival times" for each amphipod in all aquaria, except the unacidified ones, were pooled and regressed against head length and against number of antennal segments.

Chronic toxicity test

The objective was to determine the effects of moderately reduced pH (i.e. pH hypothesized to be sublethal in the short term and lethal in the long term) on survival of *H. azteca* exposed from the time of release from the brood pouch until death. Treatments (three replicates each) of 0, 1, 2, and 3 μmol H^+/L (target pH's 7.40, 6.00, 5.70, and 5.52) were established before addition of amphipods, and aquaria were maintained as described for the acute toxicity tests. Halfway through the experiment (2 wk after start), 1 L of water was removed from each aquarium and replaced with fresh lake water acidified to the target pH. Actual mean concentrations of triplicate treatments were 0.04, 1.03, 2.02, and 3.08 μmol H^+/L (= mean pH's of 7.43, 5.99, 5.69, and 5.51) (Fig. 1D). Mean pH's of treatment replicates varied ±0.025 pH unit (average 95% C.I.). Ca concentration of each aquarium was measured at the end of the experiment (Stainton et al. 1977); concentrations ranged from 2.72 to 3.31 mg/L, indicating replicable concentrations well above limiting levels (see below).

Hyalella azteca for the experiment was collected from L239 in early June 1986. Gravid females were isolated in beakers and checked at least every 3 d for offspring released from brood pouches. Sufficient amphipods for the experiment were collected and placed into treatment over four separate times between 19 and 30 June. A total of 426 amphipods <3 d old were placed into twelve 4-L aquaria of recently collected L239 water, three amphipods per vial and 12 vials per aquarium, except for one of the control aquaria, which received 30 instead of 36 animals (Fig. 1D). Vials used were the same as in the acute toxicity tests.

Amphipods were examined every 3 d until nearly all individuals in acidified treatments died, with the time of death for each individual recorded. Differences in survival among treatments within time replicates were analyzed by *K*-sample generalized Kruskal–Wallis *H*-tests (Lee 1980).

Results

Distribution of *H. azteca* at ELA

Lakes sampled for *H. azteca* ranged from pH 5.0 to 8.8, with a median of 6.6. Duplicate water samples from lakes usually differed by <0.05 pH unit, which was within the range of the measurement error. Ranges of Ca concentrations (1.1–3.5 mg/L) and Mg (0.44–1.1 mg/L) were similar to previous measurements made in the lakes (table 1 of Grapentine 1987). *Hyalella azteca* was absent from seven of the 30 lakes. Among the lakes having *H. azteca*, relative abundance was ranked as "low" in five and "high" in all others.

Comparison of relative abundance with chemical conditions of the lakes suggested that epilimnion concentrations of Ca and pH affected amphipod distribution (Fig. 2). No *H. azteca* were found in lakes with pH's < 6.0 or Ca concentrations <1.28 mg/L. Lakes in which relative abundance was ranked low had pH's < 6.9 and Ca concentrations <2.0 mg/L. The lower limit of Ca suggested in Fig. 2 (1.24 mg/L) is the average of the lowest concentration at which *H. azteca* was found, and the next lowest lake Ca concentration recorded. Similar reasoning was used in establishing the lowest pH for presence (pH 5.7, Fig. 2). Lakes were more likely to be ranked as having high relative abundances of *H. azteca* as Ca concentration and pH increased. However, *Hyalella* was absent from two lakes (117 and 661) at ≈1.60 mg Ca/L and pH ≈ 6.5, even though pH and Ca concentration were above the suggested lower tolerance levels.

Concentration of Mg at ELA did not appear to limit relative abundance of *H. azteca*. The number of lakes having high relative abundances tended to increase with increasing pH and Mg concentrations, but the two lakes with the lowest Mg

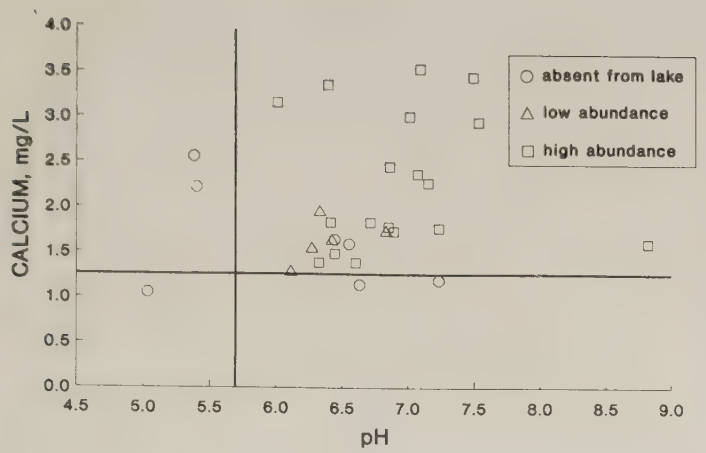

FIG. 2. pH, Ca concentration, and relative abundances of *H. azteca* in ELA lakes (see text for explanation of categories). Lines suggest lower tolerance limits.

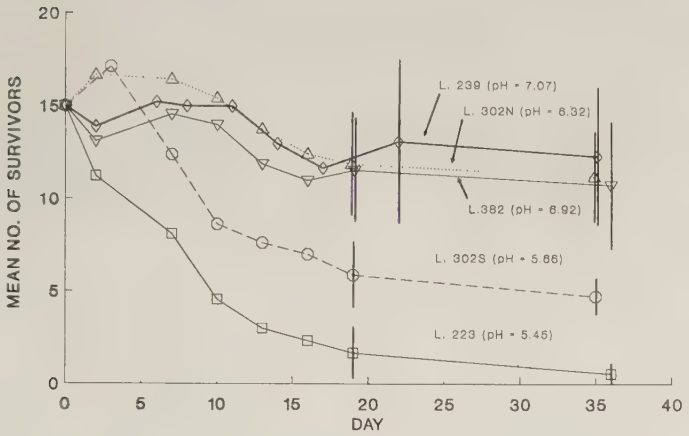

FIG. 3. Mean number of surviving *H. azteca* per enclosure in the transplantation experiment. 95% C.I.'s are shown only for the last two observations in each lake. pH values are explained in Fig. 1A caption.

concentrations (132 and 224) both had high relative abundances of amphipods. Concentration of Mg plus Ca (a rough measure of water hardness) plotted against pH did not improve discrimination among the three abundance categories over that for Ca versus pH.

Field Experiments

Transplantation experiment

Survival in unacidified L382 and L239 and weakly acidified L302N (HNO_3) was similar, and higher than survival in acidified L302S and L223 (Fig. 3). Reproduction caused initial increases in the number of amphipods in some enclosures. Survival changed little after 20 d. By day 19, variation among lakes was highly significant (ANOVA: $P < 0.001$), but variation among sites within lakes was not ($P > 0.05$). L302N, L239, and L382 had no significant differences in the number of survivors per enclosure whereas mean survival was significantly lower in L302S, and survival in L223 was significantly lower than in L302S ($P < 0.05$). After 35 d, survival per enclosure averaged 10–12 individuals in the lakes not acidified with H_2SO_4 (L302N, L239, and L382), five individuals in L302S, and less than one individual in L223.

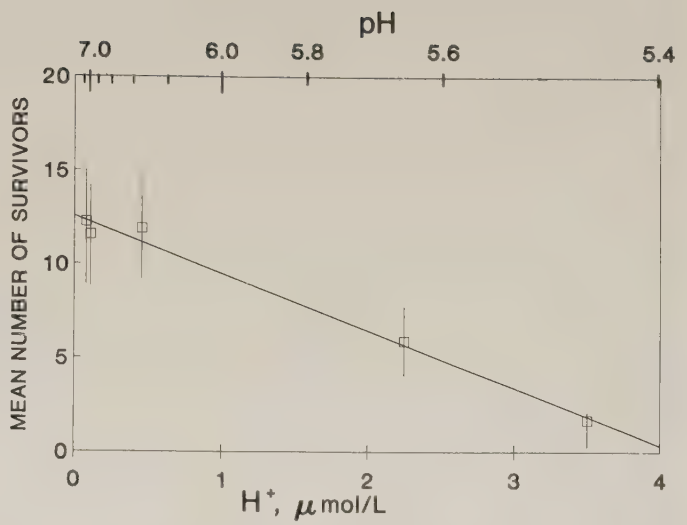

FIG. 4. Relationship between mean number of survivors of *H. azteca* per enclosure on day 19 and mean concentration of H^+ in the transplantation experiment. Error bars are ±95% C.I.

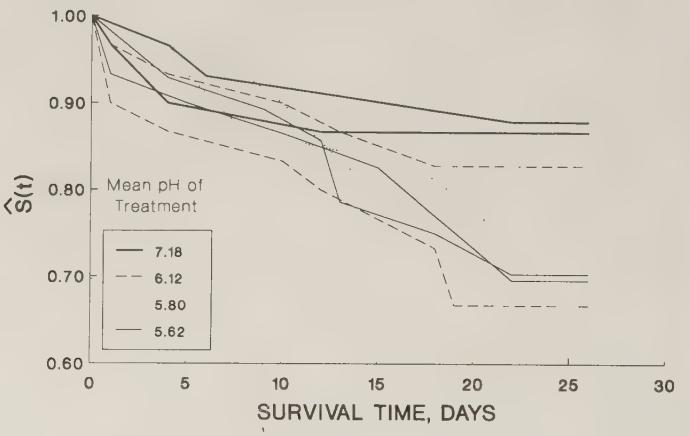

FIG. 5. Survival functions ($\hat{S}(t)$) for *H. azteca* at various pH's in the bag acidification experiment. $\hat{S}(t)$ = (number of individuals in the bag that survived longer than time t)/(total number of individuals in the bag).

An inverse, linear relationship between mean number of survivors per enclosure on day 19 and mean concentration of H^+ (Fig. 4) indicated that mean number of survivors among lakes was accounted for by differences in concentration of H^+. Concentrations of major ions among lakes, in addition to H^+, differed (D. W. Schindler, University of Alberta, Edmonton, Alta., unpubl. data), but none were related to differences in amphipod survival.

Bag acidification

Survival functions ($\hat{S}(t)$) of all amphipods in bags (i.e. size classes pooled) were similar among treatments until after 15 d (Fig. 5). By day 26, a marked decrease in survival was apparent with decreasing pH, but survival was not significantly different among treatments (ANOVA: $P > 0.05$). However, regression of survival function against mean concentration of H^+ was significant ($P < 0.05$, Fig. 6). Survival functions of treatment replicates were similar except for mean treatment pH 6.12 (pH's 6.06 and 6.17, Fig. 5 and 6). Survival in the pH 6.06 bag was much lower than that in the pH 6.17 bag due to high mortality

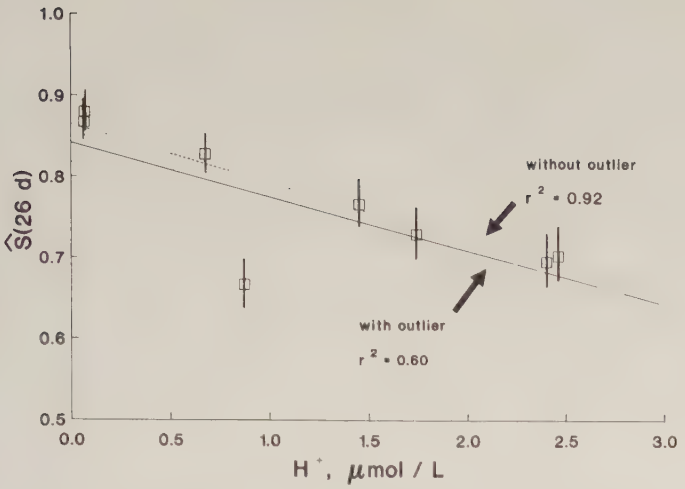

FIG. 6. Relationship between survival functions of *H. azteca* on day 26 ($\hat{S}$(26 d) ± 95% C.I.) and mean H^+ concentration in the bag acidification experiment. Linear regressions are for data with and without the outlier (bag with pH 6.06 observation).

among neonate amphipods immediately after placement into treatment (pH 6.12, Fig. 7), probably caused by handling. If survival on day 26 in this replicate of the pH 6.12 treatment is omitted, and the ANOVA recalculated, the effects of pH on survival are significant ($P < 0.01$). The regression of survival at 26 d against H^+ concentration without data from the outlier is also significant ($P < 0.01$, Fig. 6).

All size classes of amphipods survived equally well under unacidified conditions (pH 7.18, Fig. 7). Survival of neonates was significantly lower than that of adults of pH's 6.12 ($P < 0.025$), 5.80 ($P < 0.05$), and 5.62 ($P < 0.005$). Juveniles survived as well or better than adults in all treatments, except at pH 5.62.

Laboratory Experiments

Acute toxicity test

Although large differences in survival of *H. azteca* between treatment replicates were evident at pH's 5.67, 5.18, and 4.78 (Fig. 8), ANOVA of survival at 96 h indicated a significant effect of pH ($P < 0.025$). Survival in all acidified treatments was significantly lower than that of the control, and survival at pH 5.67 was significantly higher than that at pH 4.50 ($P < 0.05$). Survival at 96 h over the pH range 4.5–7.4 (0.04–32 µmol H^+/L) increased linearly ($r^2 = 0.87$, $P < 0.001$, Fig. 9).

Mortality rate of amphipods (determined as the slopes of logit *P* versus time regressions) increased in a curvilinear manner as H^+ concentration increased (Fig. 10). Estimated time to 50% mortality decreased exponentially as H^+ concentration increased (Fig. 11), and the estimated pH at which mortality reached 50% in 4 d (96-h LC50) was 5.48.

Size and age were only weakly related to survival time in acidified aquaria. Slopes of regressions of relative survival time against head length and number of antennal segments were both significantly greater than zero ($P < 0.05$), but r^2's were only 0.054 (head lengths) and 0.067 (antennal segments, Fig. 12).

Chronic toxicity test

Survival of neonate amphipods was poor in all acidified aquaria. Ten of 12 acidification replicates had no survivors after 16 d (Fig. 13). Survival also was poor in some control aquaria, indicating that stress from sources other than reduced pH caused mortality. Analysis of survival times by Kruskal–Wallis *H*-tests showed that survival was significantly different among treatments in all time replicates ($P < 0.005$). Although amphipods tended to survive longer in time replicates 3 and 4 than in 1 and 2, the same trend is evident in all time replicates (Fig. 13). Control survival times were significantly greater than those in acidified treatments within all time replicates, but differences were not significant among pH's 5.99, 5.69, and 5.51. The distributions of individual survival times (time replicates pooled), shown as percent relative frequencies of the number of days survived, differed among treatments (Fig. 14). As pH decreased, both the mean and the standard deviation of the survival times decreased from 13.7 ± 7.9 d at pH 7.43 to 5.1 ± 1.9 d at pH 5.51 (Fig. 14).

Discussion

Distribution of *H. azteca* at ELA

The geographic distribution of a freshwater species depends on historical events linked with its ability to cross barriers, its physiological requirements, and its behavior patterns (Macan 1961). The ubiquitous distribution of *H. azteca* in North America, and its apparent ease of dispersal, suggest that geographical barriers have not restricted this species from colonizing lakes. Therefore, absence from a lake implies that environmental conditions in that lake are unsuitable for *H. azteca*.

According to Wienert (1950), the most important habitat requirement for *H. azteca* is the availability of suitable organic substrate. This availability would affect distribution within a lake but probably not among lakes because such substrates exist almost universally. Normally, physical and chemical conditions in unpolluted temperate lakes are tolerable to this hardy amphipod. Competition and predation also appear unlikely to control presence of *H. azteca* in lakes (Biette 1969; Macan 1977; Crowder and Cooper 1982).

This study indicates that chemical conditions in some lakes can become limiting. *Hyalella azteca* was absent from all ELA lakes having pH < 5.7 or Ca concentration <1.24 mg/L. However, it was also absent from two lakes (117 and 661) with pH and Ca concentrations above these limits (Fig. 2), probably due to lake peculiarities. The pH of L661 was 6.44 when measured in August 1985, but pH was 5.35 in May 1985 (D. W. Schindler, unpubl. data). Substantial pH fluctuations occur in L661, probably due to variation in sulfate reduction and oxidation in the surrounding peatland (D. W. Schindler, pers. comm.), whereas in the other 20 lakes for which pH was measured (May–October 1985), seasonal variation was generally much less and pH remained above 5.7 (D. W. Schindler, unpubl. data). L117 is chemically anomalous because it has a concentration of dissolved organic carbon (DOC) of 1531 µmol C/L, which is double the average for ELA (Beamish et al. 1976). Organic anions can bind ionic Ca (Stumm and Morgan 1970; Ohle 1955a, cited in Wetzel 1975; Herring and Morel 1988), thereby making it unavailable for uptake by aquatic organisms. Because the analytical method used in this study measured total dissolved Ca and did not distinguish between free ions and Ca bound to dissolved organic anions, the amount of Ca actually available to animals in a lake with a high concentration of DOC may have been considerably less than that indicated by measurements of total Ca

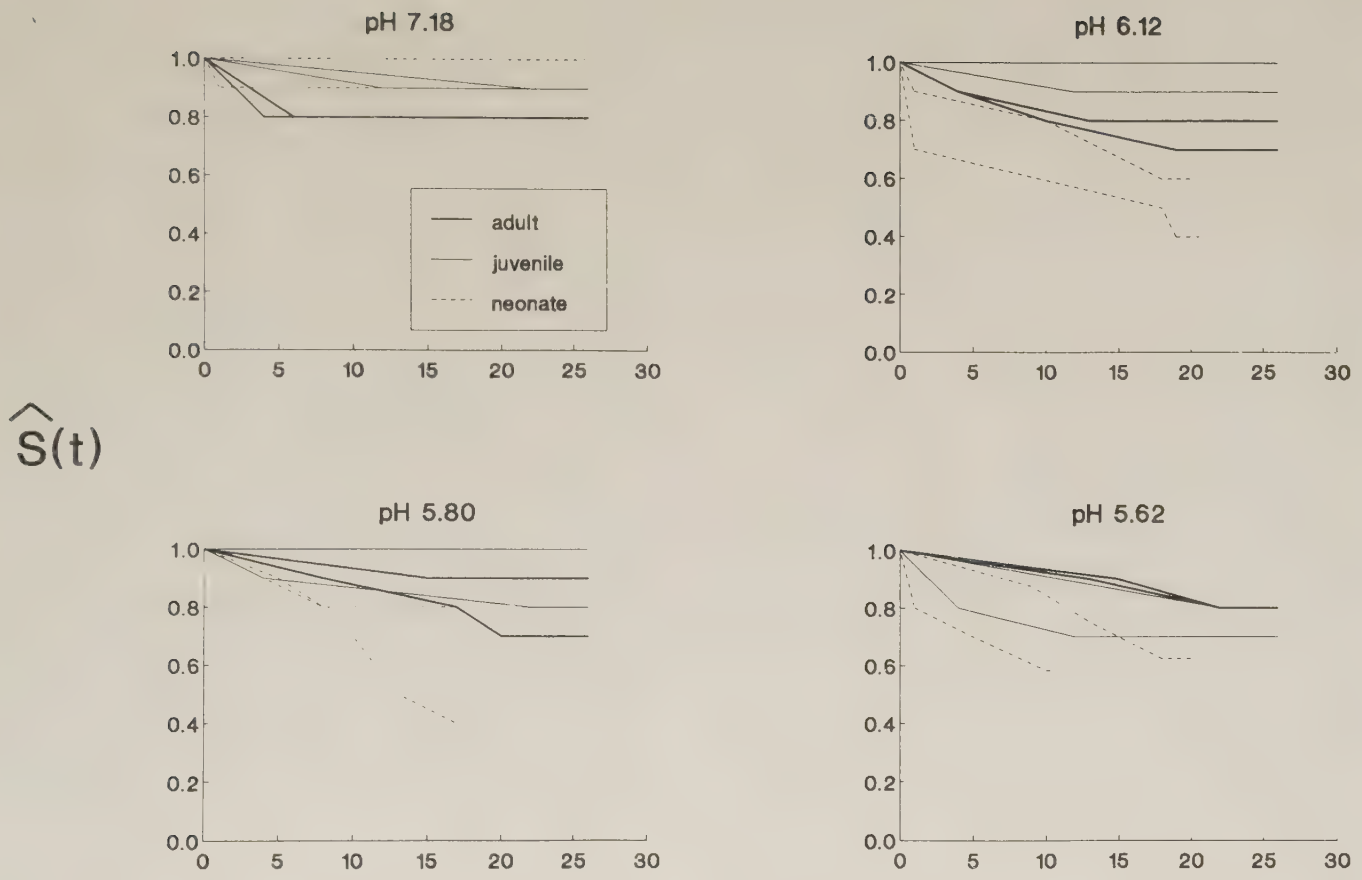

FIG. 7. Survival functions ($\hat{S}(t)$) for size classes of *H. azteca* within treatment replicates in the bag acidification experiment. $\hat{S}(t)$ = (number of individuals in the size class within the bag that survived longer than time t)/(total number of individuals in the size class in the bag). Three size classes × 2 bags = 6 functions per treatment (n = 10 amphipods per function).

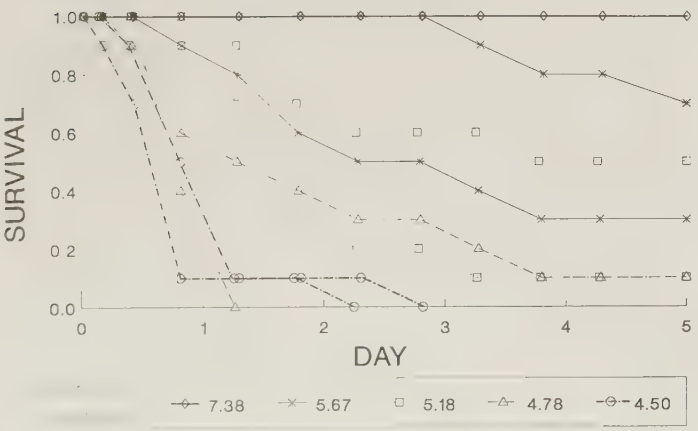

FIG. 8. Survival of *H. azteca* in the acute toxicity test. n = 10 amphipods per aquarium. Replicates of pH 7.38 had 100% survival, and overlap in the figure.

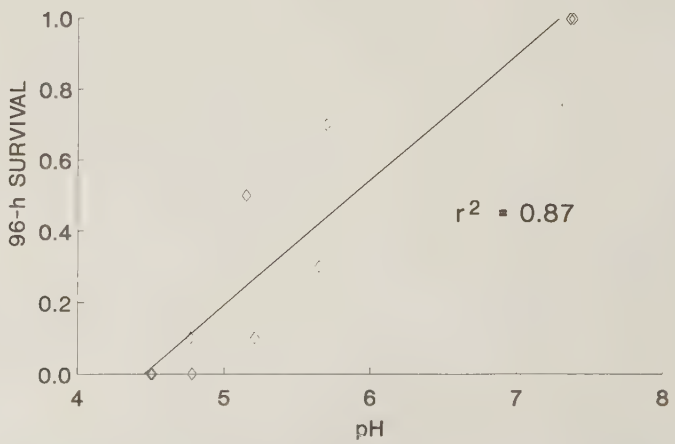

FIG. 9. Relationship between 96-h survival of *H. azteca* and pH in the acute toxicity test.

(M. Stainton, Department of Fisheries and Oceans, Winnipeg, Man., pers. comm.). Aho (1966) reported a negative correlation between the number of species of molluscs in some lakes of southern Finland and water color (a measure of the concentration of humic substances). Given equal total hardnesses, availability of Ca ions was apparently greater in colorless waters than in those rich in humic substances.

A lower tolerance limit of pH 5.7 for *H. azteca* at ELA was close to that determined by Stephenson and Mackie (1986) for *H. azteca* in southern Ontario. Of the 79 lakes they sampled,

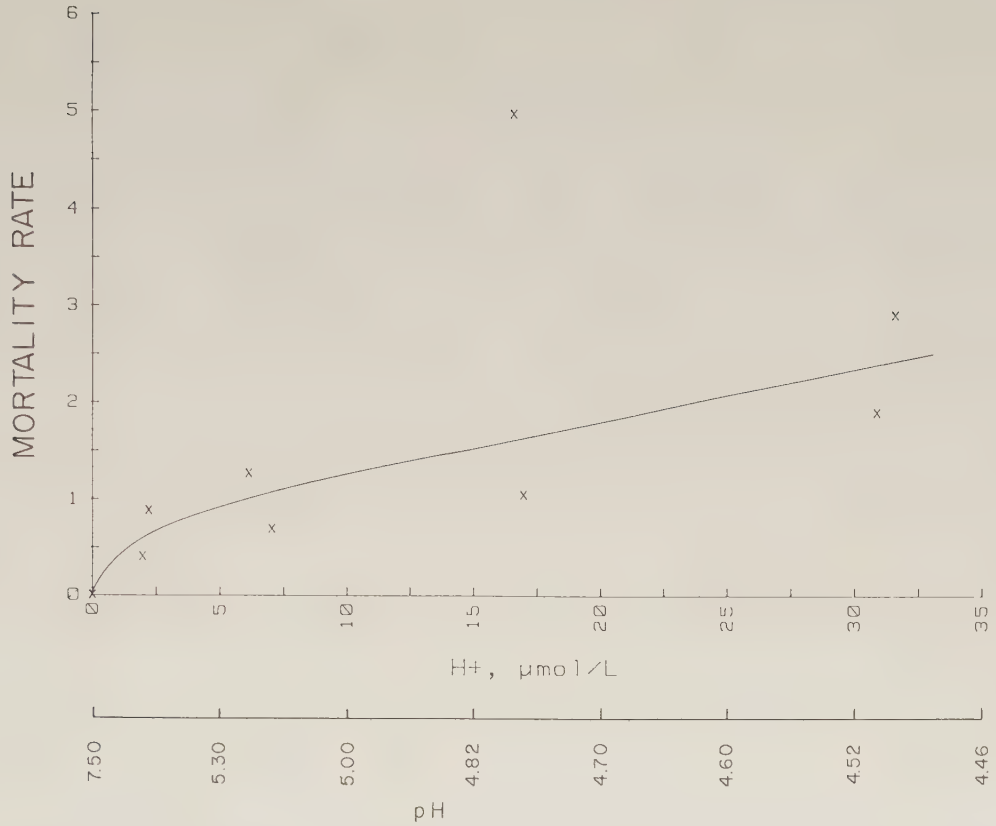

FIG. 10. Relationship between mortality rates of *H. azteca* and mean H^+ concentration in the acute toxicity test. Mortality rates are represented by the slopes of logit *P* versus time regressions (see text for details). Curve drawn by eye, excluding outlying point.

H. azteca was absent from those having negative epilimnetic summertime alkalinities (corresponding to pH < 5.6) and present in all but four of the lakes having positive alkalinities. Of these four lakes, two had prior negative alkalinities and one had low pH (5.83), low alkalinity (0.031 meq/L), and low Ca concentration (1.42 mg/L). Therefore, these lakes were vulnerable to short-term acidification below pH 5.6.

Additional evidence from two experimentally acidified ELA lakes also suggests a lower pH tolerance limit of pH 5.7. *Hyalella azteca* was collected for the first time from L223 in 1988 when the lake's mean summer pH was allowed to rise to 5.86 (M. Stephenson, Atomic Energy of Canada Limited, Pinawa, Man., pers. comm.). The species was absent in 1987, when mean summer pH was 5.45, and in all previous years during which pH was <5.45. In L302N, relative abundance of *H. azteca* declined drastically during the summer and fall of 1987, coincident with a drop in pH from >6.0 to as low as 5.4 (mean for summer = pH 5.68). During the summer of 1988 when mean pH was 5.38 (G. Linsey, pers. comm.), no *H. azteca* were collected below pH 5.6 despite repeated samplings (M. Stephenson, pers. comm.).

Not all reports agree with a proposed tolerance limit of pH 5.7. *Hyalella azteca* was abundant in the littoral zone of a poorly buffered Ontario Canadian Shield lake in which surface water pH was temporarily depressed to as low as 4.7 during spring runoff (Dermott 1985). Such pH depressions due to pulses of acidic meltwater tend to be of short duration and to occur only in the upper water layers of lakes (Jeffries et al. 1979; Hendrey et al. 1980), and in fact, the lowest pH in the top 2 cm of sediments recorded from a series of lakes, including the above one, was 5.8 (Dermott 1985). Higher pH in and near sediments is not uncommon in acidified lakes (Kelso et al. 1982; Oliver and Kelso 1983; Kelly et al. 1984) and may insulate benthic fauna from low pH in the water column (Collins et al. 1981; Dermott 1985), especially during temporary pH depressions. In addition, unlike late season depressions of pH in L661 of ELA, which occurred at water temperatures >15°C, water temperatures during spring runoff would be low (≈4°C). Stephenson and Mackie (1986) determined that at 4°C the 10-d LC50 of H^+ to *H. azteca* was pH 4.5. Thus, amphipods probably could survive a short-term depression of pH during spring runoff.

Abundance of *H. azteca* decreased with declining pH in some New Brunswick and Nova Scotia lakes, but the species was present in lakes in the lowest pH classes (4.5–5.5; Peterson 1987). Ca and Na concentrations in these coastal lakes tended to be higher than those of acidified lakes in Ontario (Peterson et al. 1986) and may have improved survival at low pH (Havas 1981). Also, pH was measured in the water column at depths of ≤5 m whereas the benthic samples were usually collected from the deepest part of the lake (Peterson et al. 1986; Peterson 1987), which may have included regions of elevated pH in or near the sediments (see above). However, it is also possible that local populations of *H. azteca* in some regions have adapted to acidic conditions.

H^+ Concentration and Survival of *H. azteca*

Increased concentration of H^+ decreased survival of *H. azteca* in field and laboratory experiments. In general, survival in acidified treatments did not become significantly lower

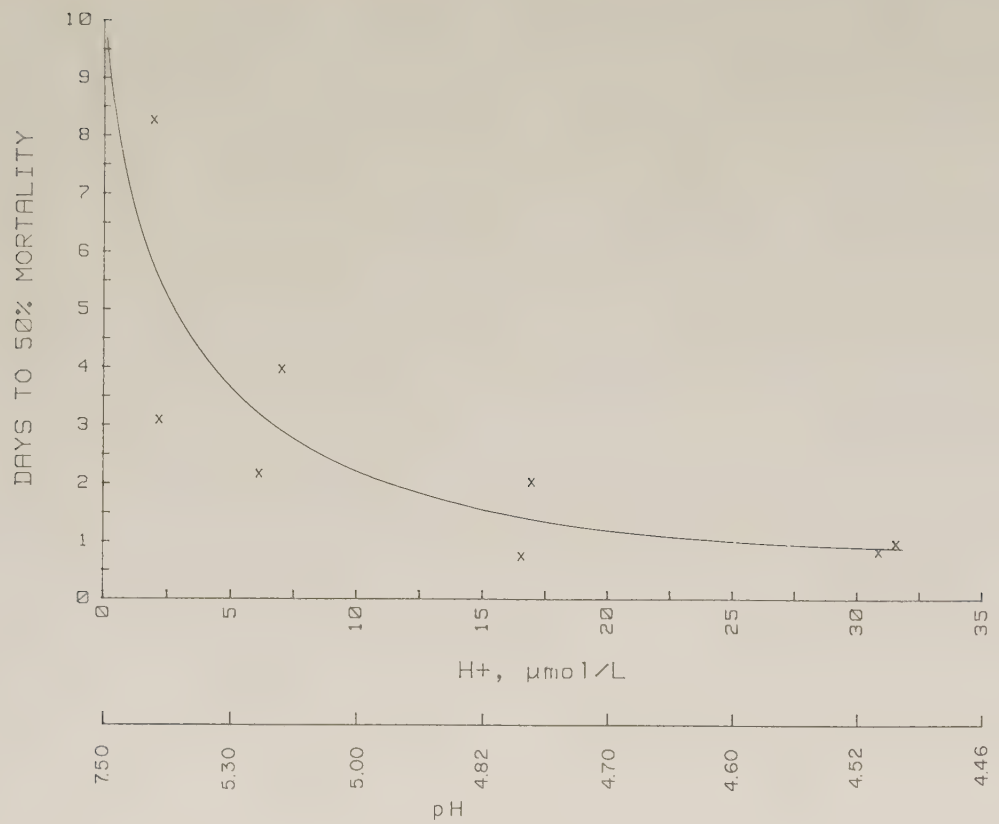

FIG. 11. Relationship between estimated time to 50% mortality of *H. azteca* and mean H^+ concentration in the acute toxicity test. Curve drawn by eye.

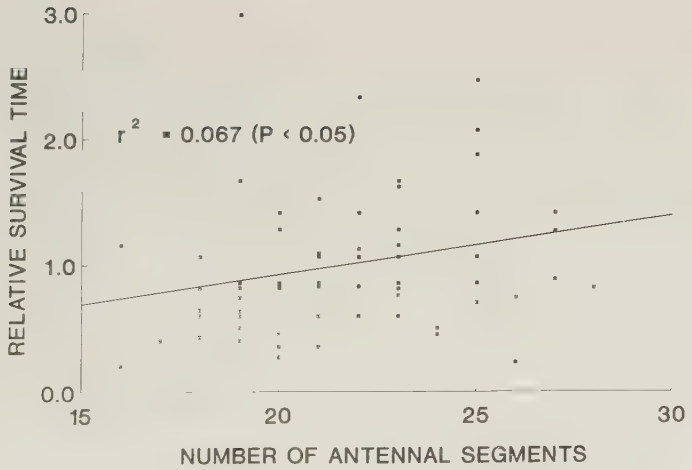

FIG. 12. Linear regression of relative survival time of individual *H. azteca* versus number of antennal segments in the acute toxicity test. Survival times of amphipods from acidified treatments (pH 4.50–5.70) were pooled after dividing by the mean survival time for the aquarium in which the amphipod was placed.

than that in control treatments until pH < 6.0. Between pH 6.0 and 5.5, survival of amphipods at a specific pH and for a defined period of exposure was variable among experiments, due to stress from handling and containment, differences in temperature, or differences in the way that populations acclimatized to dilute waters. Lifetimes of amphipods in the chronic toxicity test tended to be shorter as pH decreased; variation in survival times decreased (Fig. 14), perhaps because H^+ concentration became the major cause of mortality and overrode other factors affecting survival time. Survival of *H. azteca* was poor below pH 5.5 in all experiments. Mortality was 50% in the acute toxicity test after ≈4 d at pH 5.5 (Fig. 8); most individuals died after 3 d at pH's 4.50 and 4.78 (Fig. 11). An average of only 11% of the amphipods transplanted into L223 (pH 5.45) were alive after 20 d (Fig. 3). Survival decreased as concentration of H^+ increased in a linear manner up to at least 4 µmol/L (Fig. 4 and 6) whereas over a range of H^+ concentrations from 0.04 to 32 µmol/L, the response was linear on a pH scale (Fig. 9). These relationships suggest that the range of 0.04–4 µmol/L H^+/L (pH ≥ 5.4), over which the rate of change in response was greatest, is the most critical to survival. It is in this range that survival became significantly lower than that at neutral conditions and that long-term survival of populations became doubtful.

Once the neonate stage is passed, size and age were minor factors affecting the ability of *H. azteca* to survive acidic conditions in this study. Although amphipods <3 d old placed into acidified bags survived more poorly than adults at pH 5.62, 5.80, and 6.12 (Fig. 7), juvenile amphipods in the bags did not show significantly higher mortality than adults at any pH, suggesting that the size difference between adults and juveniles did not affect survival. At pH 4.5–5.6, size (based on head lengths) and age (based on the number of antennal segments; Fig. 12) were only slightly correlated with relative survival time of adults and juveniles in the acute toxicity test.

Survival measured at similar pH's in different experiments did not always agree, and this may have been due to differences in certain experimental conditions. For example, survival in the transplantation experiment, the least artificial of the experi-

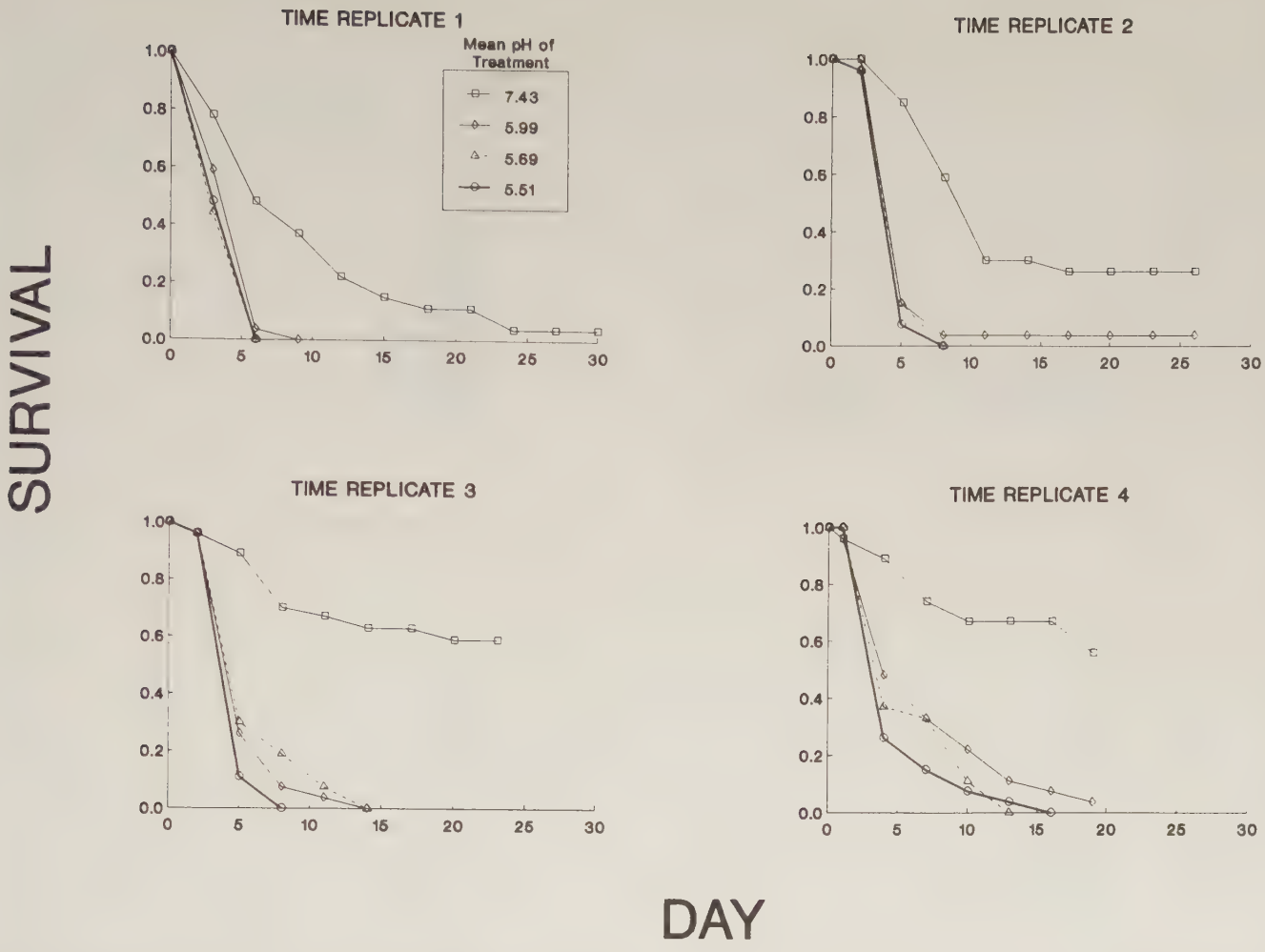

FIG. 13. Mean survival of neonate *H. azteca* within time replicates in the chronic toxicity test. Each curve is based on (3 amphipods per vial) × (3 vials per aquarium) × (3 aquaria per treatment) = 27 amphipods.

ments, was ≈40% at pH 5.66 after 20 d whereas in the bag acidification experiment, survival was 70% at pH 5.62 after 20 d. However, water temperatures and chemicals added were different in these two experiments (20–27°C for the transplantation experiment versus 14–20°C for the bag acidification experiment; H_2SO_4 acidification in L302S and L223 versus HCl and NaOH in the bags). Lower temperatures can reduce osmoregulatory stress caused by acidified waters because turnover rates for water and ions decrease with temperature (Sutcliffe 1978). The presence of high concentrations of Na^+ can lessen the loss of Na^+ and thus partially alleviate the osmotic stress of low pH (Meinel et al. 1985). However, differences in temperature rather than Na^+ concentration probably were more important in explaining differences in the two experiments because the protection offered by Na^+ occurs at considerably higher concentrations than were present in the bags (Meinel et al. 1985).

Survival in laboratory experiments was generally lower than in the field experiments, perhaps due to containment and handling effects. In the acute and chronic toxicity tests, survival ranged from 30 to 50% at pH ≈ 5.7 after 5 d whereas in the acidified lakes and bags, survival was 85–95% after 5 d at pH 5.6–5.8. Amphipods in laboratory experiments were held under conditions that simulated midsummer in the littoral zones of ELA lakes. Although concentrations of Ca^{2+} were not depleted after 30 d, it was possible that another required substance became depleted or that a contaminant accumulated, which would have had a greater effect in 4-L laboratory aquaria than in the 500-L bags. Also, amphipods were handled more frequently to assess survival in the acute toxicity test than in the other experiments (12 times over 5 d versus ≤3 times over 5 d), and this could have reduced survival, especially that of neonates.

Increased sensitivity to low pH would be expected of neonates because, compared with older amphipods, neonates have higher metabolic rates, molt more frequently, and have greater demands for essential ions and nutrients, all of which increase sensitivity of young to environmental stresses (Buikema and Benfield 1979). However, H^+ concentration alone does not account for the poor survival observed in the chronic toxicity test. Survival of neonate amphipods at pH 6.0 only ranged from 15 to 43% at 5 d and from 0 to 22% at 10 d (mean survival within time replicates). These values are much lower than results from the bag acidification experiment, in which survival of neonates was >60% after 10 d at pH as low as 5.62 (Fig. 7). Survival in the chronic toxicity test was also poor under control conditions (Fig. 13), so factors other than H^+ concentration probably contributed to mortality in the acidified treatments.

Implications for Natural Populations

Information from all of the experiments detailed above and the ELA distribution data can be used to derive an estimate of

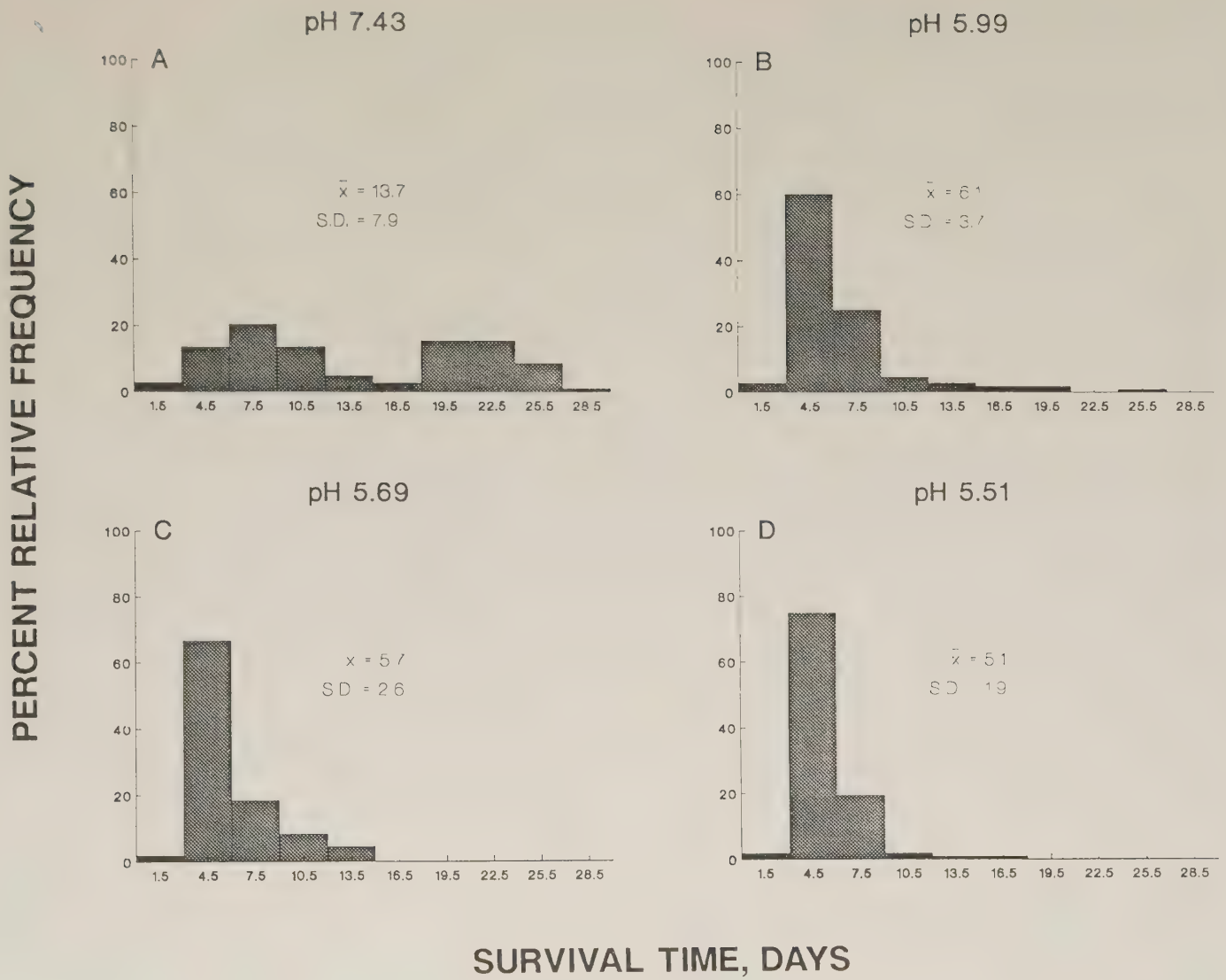

FIG. 14. Distributions of survival times of *H. azteca* in the chronic toxicity test. $n = 108$ for all treatments except pH 7.43, for which $n = 102$.

the minimum pH (or highest H^+ concentration) that would allow long-term survival of natural *H. azteca* populations. It is possible to determine, for each of the four approaches used in this study, the highest pH at which survival is significantly lower than that at neutral pH and then use these values to derive a common or composite estimate of critical pH. This estimate represents the point at which stress from the toxicant is first detected in a population, but it is probably higher than the concentration (micromoles per litre) at which growth, reproductive output, behavior, and various physiological functions are affected. In fact, use of the survival data in this way is conservative. Mayer et al. (1986) analyzed endpoints such as survival, growth, reproduction, various physiological characters, and behavioral traits for 28 chemicals and seven fish species in 34 chronic toxicity tests and determined that the "no observable effects concentration" (NOEC) for survival, which is the highest observed concentration of a toxicant that shows no significant difference from the responses measured in a control treatment (Maki 1979; Capizzi et al. 1985), was up to five times the NOEC's for all other endpoints in ≈95% of the comparisons.

Responses of *H. azteca* to concentrations of H^+ for each approach used in this study can be summarized in terms of survival, or presence in a lake (a gross measure of survival), and whether or not either one differs from the response shown at control or neutral-pH conditions (Fig. 15). In the distribution study, presence in the lake is the response at neutral pH whereas absence is the significantly different response, although not in a statistical sense. Historical data for L223 and L302N (M. Stephenson, pers. comm.) and L302S (L. C. Grapentine, unpubl. data) also involve presence/absence. Some straightforward assumptions have been made in interpreting these data. For example, in 1983, *H. azteca* was present when the mean epilimnion pH of L302S during the ice-free period was 5.9, and this was assumed to apply to previous years when the lake was unacidified. In 1984, when the pH had decreased to 5.6, *H. azteca* was absent, and this was considered to be a significant departure from the neutral state. For the acidification experiments, significantly different survival from that in control treatments was determined by several statistical methods (see above).

Overall, it appears that below a pH of 5.85 (1.4 μmol H^+/L), responses become significantly different from those of

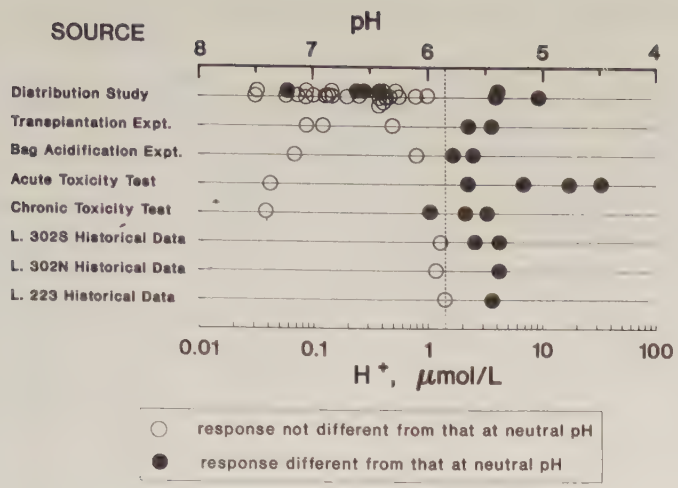

FIG. 15. Responses of *H. azteca* to concentrations of H^+ for each approach used in this study. Broken line indicates the pH below which responses appear to become significantly different from neutral or control conditions (see text for details).

the neutral condition (Fig. 15). Some exceptions should be noted. In the distribution study, *H. azteca* was absent from four lakes (117, 118, 220, and 661) having pH > 6.0 (Fig. 2). Reasons for L117 and L661 were discussed above. Absence from L118 and L220 probably was due to Ca concentration <1.24 mg/L. In the chronic toxicity test, survival of neonate amphipods at pH 6.0 was significantly lower than that in control treatments, probably because of handling and container stress. The results shown in Fig. 15 suggest that long-term survival of *H. azteca* is impaired when pH remains <5.8.

Inferring a pH tolerance limit for natural populations of *H. azteca* is constrained by factors such as the effect of sediments on the pH of overlying water, temperature, and concentrations of Na^+ and Ca^{2+}. These factors may mask or confound the expected relation between the distribution of *H. azteca* and pH (see above). Trace metal concentrations in acidified lakes also can interact with H^+ effects on *H. azteca*, but little information exists on the toxicity of metals to *H. azteca* in acidified waters. In general, concentrations of metals that are toxic to amphipods in laboratory studies tend to exceed concentrations encountered in acidified waters, except for lakes receiving direct fallout from smelters or drainage from acid mines (details in Grapentine 1987).

Responses to acidification shown by *H. azteca* from northwestern Ontario (ELA) compare well with those observed by Stephenson and Mackie (1986) for southern Ontario, although they suggested a critical pH of 5.6 and our study indicated pH 5.8 as a lower limit. However, the estimate from our study may be conservative because it was based on (a) presence/absence data from a series of lakes that did not include pH's between 5.4 and 6.0, (b) experimental data showing significantly lower survival at pH ≤ 5.8, which does not necessarily mean that extinction is imminent, and (c) a conclusion that required the least number of assumptions about the sensitivity of *H. azteca* to H^+.

Because of its sensitivity to pH, *H. azteca* is useful as an early indicator of acidification. The species is ubiquitous, so its absence or disappearance from a culturally acidified lake is unnatural. In addition, its high potential for dispersal should allow early recolonization of lakes recovering from acidification. Therefore, we support the suggestions of Stephenson and Mackie (1986) and Mills and Schindler (1986) that *H. azteca* be included in programs monitoring the effects of acidification in North America.

Acknowledgments

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A Paleolimnological Assessment of Acidification and Liming Effects on Diatom Assemblages in a Swedish Lake

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In Sweden, about 6000 lakes have been limed to mitigate acidification. Lysevatten (southwestern Sweden) was limed in 1974 and 1986 and has better historical pH and fish records than most other, similar forest lakes. Historical data were compared with diatom analyses of a sediment core to assess to what extent diatom-inferred pH reflects the known pH history. The diatom-inferred pH history agrees well with the development known from documentary sources, although the sediment record is slightly smoothed. Both the historical and sediment records show that the acidification, with acid episodes that caused severe damage to lake biota, began during the 1940s and became acute in the 1960s, with pH values of 4.5–4.9. Prior to acidification, lake water pH was between 6 and 7, and the lake had a planktonic *Cyclotella* flora. The liming increased pH to 7.5 and resulted in the expansion of *Achnanthes minutissima* agg., *Cymbella microcephala*, and *Synedra acus*. The reacidification is reflected by a decrease of the abundance of these species. Neither the liming in 1974 nor that in 1986 restored the preacidification diatom flora; there was no recovery of *Cyclotella*.

Environ 6000 lacs de Suède ont été chaulés afin de lutter contre l'acidification. Le lac Lysevatten, situé dans le sud-ouest du pays, a été chaulé en 1974 et en 1986 et l'on possède, pour ce lac, de meilleurs relevés historiques du pH et de la faune ichtyologique que pour la plupart des autres lacs semblables en milieu forestier. Les données historiques ont été comparées aux résultats d'analyses des diatomées d'une carotte sédimentaire afin d'évaluer la mesure dans laquelle le pH déterminé à partir des diatomées reflétait l'évolution connue du pH dans le temps. Les valeurs du pH déterminées à partir des diatomées correspondent bien à celles des données historiques bien que l'on note un certain lissage de la courbe. Les deux séries de données montrent que l'acidification, dont certains épisodes ont causé d'importants dommages du biote du lac, a débuté au cours des années 1940 et est devenue aiguë au cours des années 1960, lorsque le pH s'est situé dans la gamme des 4,5–4,9. Avant l'acidification, le pH du lac était de 6 ou 7 et la flore planctonique lacustre était de type *Cyclotella*. Le chaulage a permis d'accroître le pH à 7,5 et a donné lieu à une expansion de *Achnanthes minutissima* agg., de *Cymbella microcephala* et de *Synedra acus*. La réacidification s'est traduite par une baisse de l'abondance de ces espèces. Les chaulages de 1974 et de 1986 n'ont pas permis à la flore de diatomées d'avant l'acidification de se rétablir; les *Cyclotella* ne se sont pas rétablies.

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In Sweden, 16 000 lakes are acidified (Brodin 1990a) and prior to 1990 about 6000 of these had been limed at least once (Brodin 1990c). In other countries, liming as a measure to counteract acidification is less common, but a number of liming experiments and smaller programmes have been launched (see Brocksen and Wisniewski 1988; Olem 1991). Although studies of the effects of liming have been made since this practice became common in the 1970s in Sweden (Lessmark and Thörnelöf 1986; Nyberg and Thörnelöf 1988), effects on lake ecosystems are not fully understood (Weatherley 1988; Gahnström 1989). One reason for this is that it takes several years just to monitor the development from the actual lime treatment until the most obvious effects of the liming on lake chemistry and biota cease. Paleolimnological investigations made several years after the liming are therefore a convenient method to study certain aspects of liming. A paleolimnological approach also provides background information about conditions in lakes before and during the acidification period preceding liming, which is necessary background information for the evaluation of the liming effects.

This paper discusses Lysevatten, a lake in southwestern Sweden, which has been limed twice, the first time in 1974 (Hultberg and Andersson 1982). There are good historical pH and fish records for Lysevatten, which show that the lake has gone through the following phases during this century: acidification, liming, reacidification, and reliming. We compare these historical records with records of diatoms in sediment cores to assess how well the subfossil diatom record reflects the known recent pH trends of the lake (mainly post-1960) and use the sediment record to study the earlier acidification history and to evaluate changes in diatom assemblages caused by acidification, liming, and reacidification. Of particular interest was to assess whether the liming restored the preacidification flora of the lake.

Site Description and Historical Record

Lysevatten (58°04′N, 12°02′E) is situated in Bohuslän, southwestern Sweden, about 50 km north of Göteborg. The lake area is 0.39 km^2, maximum depth is 18.5 m, mean depth is 4.7 m, and the retention time is 2.5 yr. The catchment area is 1.46 km^2. The lake is surrounded by forests of mainly Norway spruce, Scots pine, and birch, which have been partly clear-felled during the last decades. The bedrock is granitic, soils are generally thin, and outcrops of bedrock are common. The lake lies very close to Lake Gårdsjön and more details about this area of high acid deposition are presented in the two volumes on the Gårdsjön Project (Andersson and Olsson 1985; Dickson 1988).

In July 1900, F. Trybom visited Lysevatten and he noted that the lake supported perch (*Perca fluviatilis*), northern pike (*Esox lucius*), European eel (*Anguilla anguilla*), and a rich population of roach (*Leuciscus rutilus*) (Trybom 1903). Fishing with seine-net took place in the lake. Roach had been stocked in 1880. In September 1948, N. Törnquist found the same species but noted that the populations of northern pike and roach were sparse (Törnquist 1948–49). Measured pH was 6.0.

From April 1961 to March 1962, Alm and co-workers performed an intense water sampling programme in Lysevatten and neighbouring lakes (Alm 1961–62). They recorded pH values below 5 at snowmelt in both these years as well as during the rains in November 1961. In summer 1961, however pH was above 6 (see Fig. 5).

Hultberg and co-workers monitored the lake from 1970 to 1973 and recorded summer pH values between 4.4 and 4.9. Survey fishing during that period and a rotenone treatment in 1973 produced only about 100 specimens of perch in total and no other species (Hultberg 1985).

The lake was first limed in April 1974 when 60 tonnes of $CaCO_3$ was spread from a boat. The pH immediately increased to about 7.5 (see Fig. 5). Following liming, the lake was stocked with trout in 1975. The lake gradually reacidified and a second liming was done in November 1986 by spreading 100 tonnes of ground limestone using a mobile raft.

Material and Methods

The main core (LV1985) was taken in March 1985 and was used for relative diatom analysis and analysis of spheroidal carbonaceous particles from fossil fuel combustion. Since no sediment was left when a later decision was made to date the sediment by ^{210}Pb, another core (LV1986) was taken in February 1986. Together with dating, quantitative and relative diatom analyses and carbonaceous particle analysis were made on this core, the two latter analyses to provide data for a correlation of LV1986 and LV1985. Some comparison is also made with a core from 1979 (LV1979) (Renberg and Hellberg 1982). A surface sediment sample, obtained in September 1987 with a Kajak sampler, is also discussed.

The sediment cores were taken with a freezer corer (Renberg 1981) from the deepest part of the lake. LV1985 and LV1986 were taken, however, about 20 m apart to avoid disturbing an in situ experiment undertaken in the lake in 1986. The frozen cores were subsampled, following methods described by Renberg (1981), into contiguous subsamples: 0.25 cm thick for diatoms and 0.5 cm thick for carbonaceous particles in LV1985, 1.0 cm thick for all analyses in LV1986, and 0.5 or 1 cm thick in LV1979.

Microscopic slides for relative diatom analysis were done using hydrogen peroxide cleaning and mounting in Hyrax or Microps (Renberg 1990a). About 400 diatom valves were counted on each level. The diatoms were classified into Hustedt's pH categories following Renberg (1976) and Renberg and Hellberg (1982). Diatom-inferred pH values were obtained with index B (Renberg and Hellberg 1982) and weighted averaging (Birks et al. 1990b); index B reconstructions were done to allow comparisons with earlier studies from this area. Quantitative diatom analyses for calculations of net annual accumulation values were done with the microsphere addition method (Battarbee and Kneen 1982). Diatom nomenclature follows Williams et al. (1988). The computer program Polldata (Birks and Huntley 1978) was used for calculations and construction of diatom diagrams.

Spheroidal carbonaceous particles, which are good indicators of atmospheric deposition of pollutants from fossil fuel combustion (Wik and Natkanski 1990), were counted by M. Wik using the method described by Renberg and Wik (1985b).

^{210}Pb analyses were done by P. Appleby and F. Oldfield, University of Liverpool, using gamma spectroscopy (Appleby et al. 1986).

Results

All frozen cores of Lysevatten show discrete but clear stratigraphic changes in sediment colour. From being chocolate brown at the bottom of the cores (LV1985, LV1986), the sediment becomes slightly darker at 11–12 cm and then lighter brown again at 3–4 cm below the sediment surface (Fig. 1). The organic matter content is high (60%) and increases slightly up-core with a peak (75%) at about 3 cm. The mineral grain content of the sediment is very low; the residue after ignition was mainly diatom valves and chrysophyte statospores.

The carbonaceous particle profiles (Fig. 1) show a pattern typical for Swedish lakes (Renberg and Wik 1985a). There is a weak but detectable increase in carbonaceous sphere concentration of the sediment following the industrialization in the late nineteenth century, a marked increase starting with the industrial upturn after World War II, reaching a maximum about 1970 prior to the oil crisis, and decreasing values to the present.

The ^{210}Pb chronology for LV1986 presented in Fig. 1 is the mean of the dates given by the CRS and CIC dating models (Appleby and Oldfield 1978). There was very good agreement between the two models showing that there have been no major changes in sediment accumulation rates over the past 130 yr. For sediments postdating 1900, the CIC dates were virtually identical to the CRS ^{210}Pb dates. For sediments predating 1900, discrepancies between the two models were within the standard errors. Further credence of the chronology is given by a peak in the sediment at 3.5 cm (1963) of ^{137}Cs derived from atmospheric nuclear weapons testing.

Already the colour variations with depth indicate that the stratigraphy of the cores from the two different years was not directly comparable in detail. A reliable correlation and transfer of the ^{210}Pb dates from LV1986 to LV1985 was achieved, however, by carbonaceous sphere and diatom analyses (Fig. 1).

About 110 diatom taxa were found in the master core (LV1985). There are marked stratigraphic changes in the species composition and the relative frequency of many species (Fig. 2). The lower part (5–20 cm) is dominated by two planktonic *Cyclotella* species, *C. kuetzingiana* and *C. glomerata*, the latter with a pronounced peak between 12 and 16 cm. Abundant

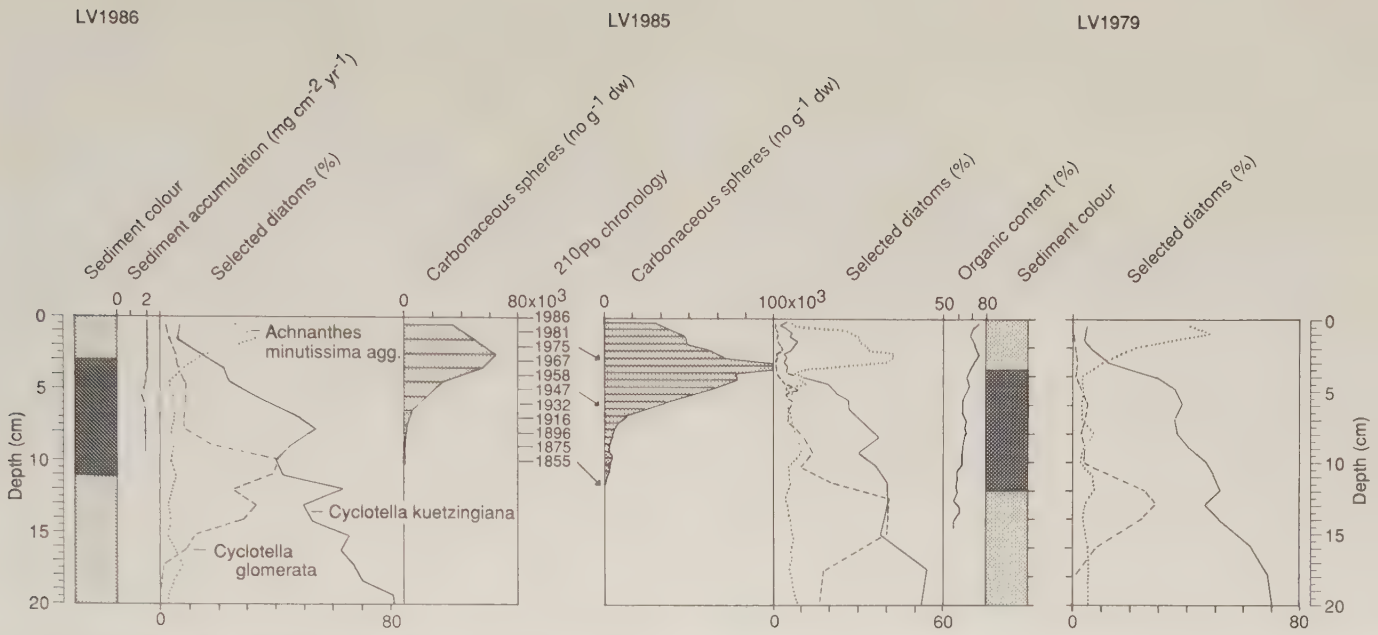

FIG. 1. Some sediment characteristics, relative frequency of some important diatom species, and carbonaceous sphere concentrations in cores LV1986 and LV1985 from Lysevatten. The analyses of LV1986 were used to transfer ^{210}Pb dates from this core to the master core (LV1985). The diatom analyses from the core taken in 1979 (LV1979) support the supposition that the stratigraphy of the master core (LV1985) is representative and reliable, but show also that a detailed correlation is required when results from one core are used for interpretation of results from another core due to small but significant depth–time differences in different cores.

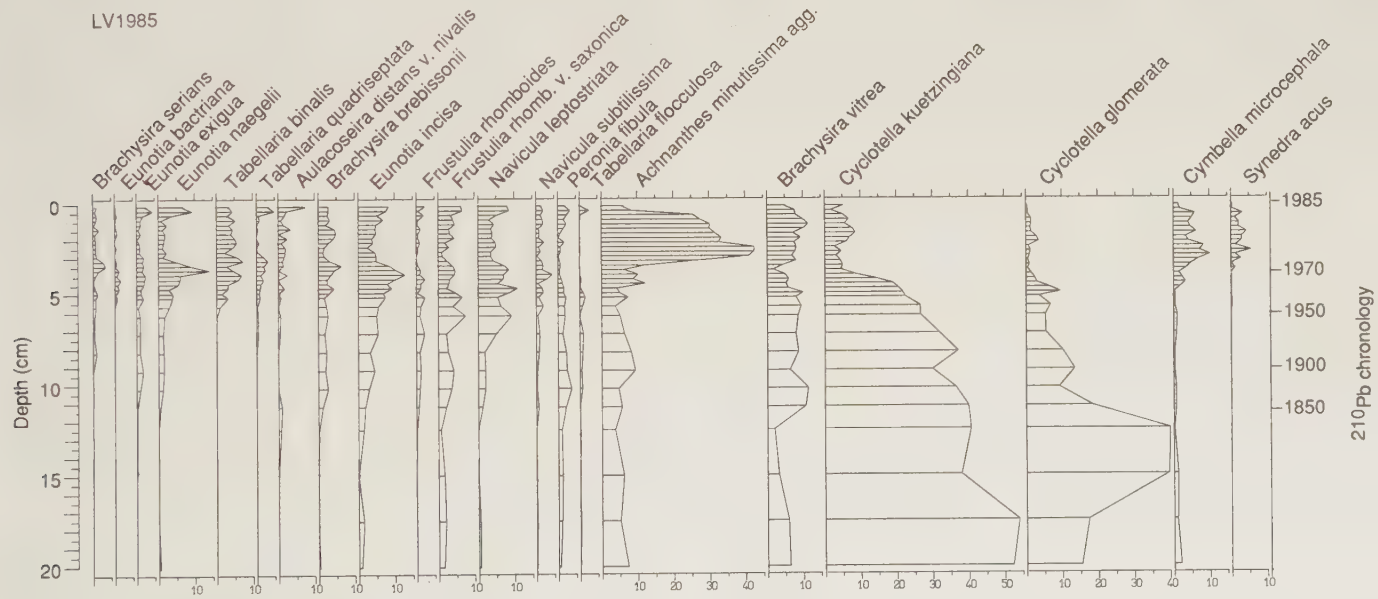

FIG. 2. Relative frequency (%) of selected diatom taxa in master core LV1985 from Lysevatten.

diatoms in the upper 10 cm and with maxima between 3.5–7 cm are periphytic taxa such as *Brachysira brebissonii, Eunotia incisa, Frustulia rhomboides* and var. *saxonica, Aulacoseira distans* var. *nivalis, Navicula leptostriata, Peronia fibula, Tabellaria flocculosa, Navicula subtilissima, Tabellaria binalis, Tabellaria quadriseptata, Brachysira serians, Eunotia exigua*, and *Eunotia naegelii*. *Achnanthes minutissima* agg., *Cymbella microcephala*, and *Synedra acus* are abundant in the top 3.5 cm of the core.

The relative frequency of diatoms classified as acidophilous and acidobiontic begins to increase at about 11–12 cm depth (Fig. 3). This trend is abruptly broken at 3.75 cm by the increase of indifferent (= circumneutral) and alkaliphilous taxa, and then acidophilous taxa once again gradually begin to increase their relative abundance. Inferred pH values match this picture; values obtained by weighted averaging fall from about 7.1 to 5.1 and increase to about 6.3 after the liming and decrease to 5.1 at the surface of the core (Fig. 3). Index B inferred values show the same trend but values are slightly lower.

The net annual accumulation rate of diatom valves at the sampling site has changed markedly during the period covered by core LV1986 (Fig. 4), although the sediment accumulation

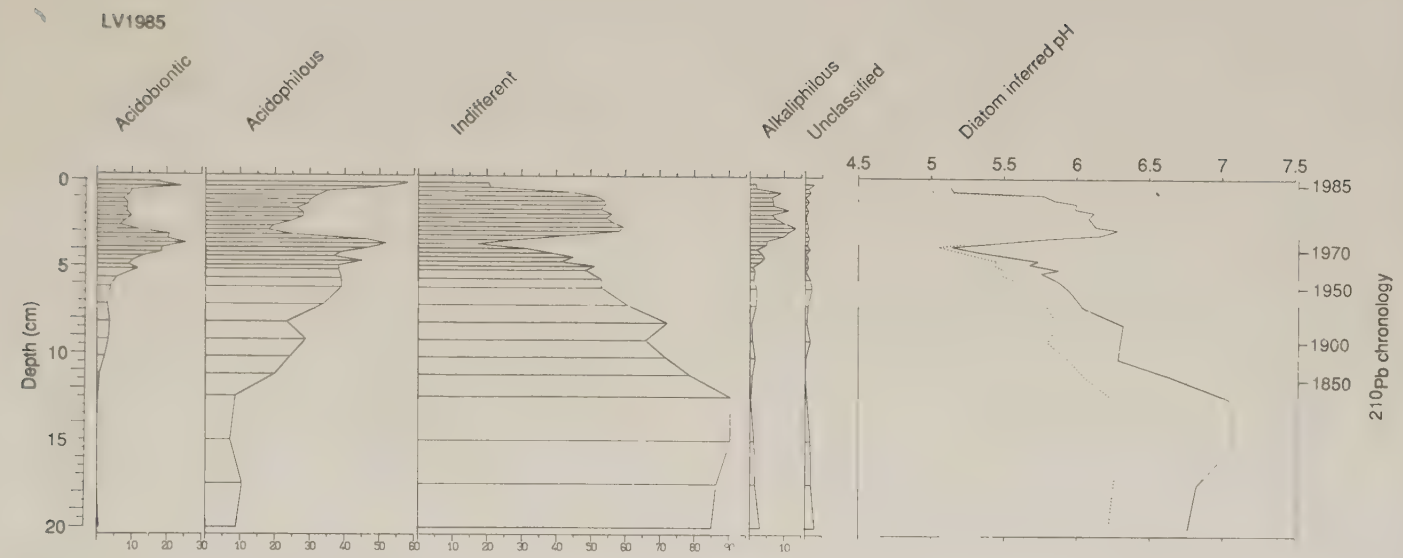

FIG. 3. Diatoms in LV1985 classified into Hustedt's pH categories (%), and diatom-inferred pH using weighted averaging (solid line) and index B (broken line). Root mean squared error of prediction for weighted averaging calibration is 0.3.

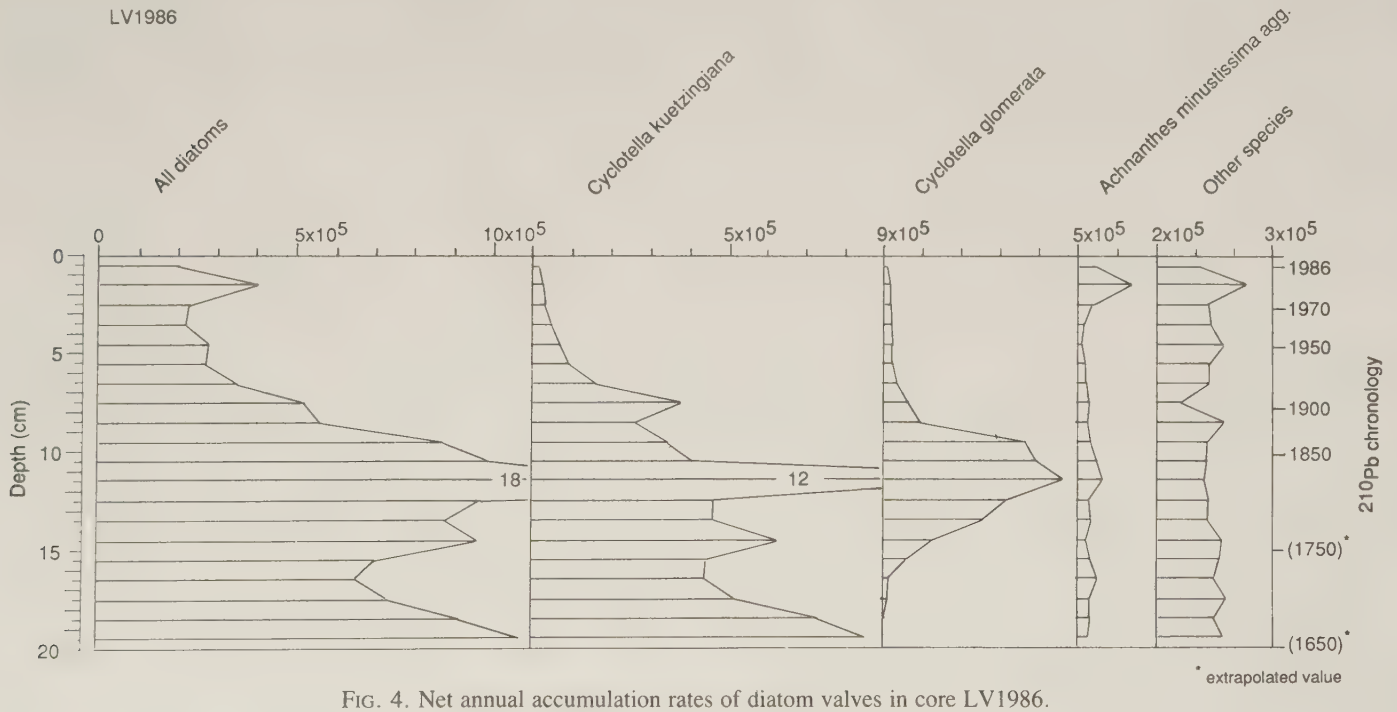

FIG. 4. Net annual accumulation rates of diatom valves in core LV1986.

rate (2 mg·cm^{-2}·yr^{-1}) has been almost constant (Fig. 1). In the lower part, before acidification began, about 1 million valves·cm^{-2}·yr^{-1} were accumulated, while later rates declined to about 0.3 million valves. *Cyclotella kuetzingiana* accounts for a considerable part of this decrease, as it decreased from about 0.5 to 0.05 million valves·cm^{-2}·yr^{-1}. Increased accumulation of *Achnanthes minutissima* agg. occurred near the top of the core.

Discussion

Comparison between Documentary and Paleolimnological Records

Important for the comparison between the historical and sediment records is the quality of the different records. The consistency of the diatom stratigraphies of the three cores LV1979, LV1985, and LV1986 (Fig. 1), although subsampling intervals differ, clearly indicates that the master core LV1985 is representative. Although Lysevatten has good historical data compared with most lakes, the documentary record before 1960 is poor and it is mainly for the post-1960 period that a detailed comparison can be made.

The lake water sampling program and the fishery data show that pH deteriorated before the 1960s; in 1961–62, acid episodes occurred in both spring and autumn. A permanent pH of about 4.4–4.9 was reached at, or even before, 1970. This acid period is reflected in the diatom stratigraphy by high frequencies of acidophilous and acidobiontic taxa. The liming of the lake in 1974 increased the frequency of indifferent (circumneutral) and

alkaliphilous taxa. The reacidification is recorded by increasing percentages of acidophilous and finally acidobiontic taxa (Fig. 3), and the second liming in 1986 again resulted in high values of indifferent and alkaliphilous taxa (not shown in the diagram). It is obvious, therefore, that the recent pH history is recorded in the subfossil diatom record. There is not, however, a perfect correlation as illustrated by both the pH category diagram and the reconstructed pH values (see Fig. 3 and 5). The discrepancy between the known pH history and the diatom-inferred pH values involves at least three factors: (1) bioturbation and physical mixing, (2) redeposition of older sediments from shallower water, and (3) no analogues for the postliming flora in the calibration data sets.

Since the sediment is not varved, there is obviously some bioturbation or other mixing of the sediment which has contributed to the smoothing of the curves. With the liming of the lake, pH changed from 4.5 to 7.5 literally from one week to the next. From limnological investigations (Lazarek 1986; Roberts and Boylen 1989), it is known that the abundance of *Achnanthes minutissima* agg. (incl. *A. microcephala*) increases markedly within a month or a year after liming, and the pattern of the *Achnanthes* curve in the sediment core can thus provide information about the vertical mixing rate. In Lysevatten the increase is very steep (Fig. 2), which indicates that the mixing is not a substantial problem. The vertical zone affected by some mixing, 0.75 cm in LV1985 (including the error caused by subsampling), corresponds to not more than about 5 yr.

Secondary sedimentation of matter resuspended from the littoral zone may be a more important factor, at least for some parts of the core. During the acid period prior to the liming in 1974, about 5% of the diatom assemblage in the core was *Cyclotella kuetzingiana*, a circumneutral species requiring pH values above 5.5–6.0 (Battarbee 1984; Charles 1985; Hörnström et al. 1985). In a core from the adjacent Lake Gårdsjön, Renberg et al. (1985) recorded a *Cyclotella* accumulation rate for the acid period that was 10–40% of preacidification values and suggested that this was due to redeposition and upward biotransport of older diatom valves. In Lysevatten, acidophilous and acidobiontic diatoms are still very frequent (>30%) in the sediment deposited after the liming. It is unlikely that all these diatoms could have grown in the lake after the liming, although the water of the littoral zone also in limed lakes can become very acid, particularly in spring (Hasselrot et al. 1987; Henrikson 1988). It is more likely that resuspension of diatom valves from shallower areas caused most of the accumulation of these diatoms. The liming caused degeneration of algal and *Sphagnum* mats which were built up during the acid period (Grahn and Sangfors 1988) and into which fine sediment material was incorporated (Andersson 1985). This degeneration process led to gradual transport of material from the littoral to the profundal zone (cf. Wik and Renberg 1991). It is therefore possible that the liming enhanced accumulation in the profundal zone of diatoms from the preliming period and contributed significantly to the smoothing of the sediment record in the postliming period.

The liming created an "artificial" diatom flora of the lake (see below). This flora has no analogue in either the calibration data set used for development of the index B method ($n = 30$) (Renberg and Hellberg 1982) or in the Surface Waters Acidification Project data set ($n = 170$) (Stevenson et al. 1991) used for calibration of the weighted averaging technique (Birks et al. 1990b). This factor, and particularly the mixture of a liming flora and an acid flora, must restrict the possibilities to make a perfect pH reconstruction using existing methods. Moreover, the pH fluctuations in Lysevatten have been extremely large over a very short period. These pH changes could not even in theory be expected to be perfectly reflected by the sediment record (see below). Given all these facts, the results obtained are rather good and could perhaps have been even slightly better if samples less than 2.5 mm in thickness and representing a smaller area of the lake bottom (here 3 cm^2) had been analysed (a large area increases the risk that a very thin sample slice cuts over sediment deposited over a longer time than a small area).

Acidification History and Effects of Liming

The 20-cm-long core LV1985 reaches about 300 yr backwards in time (extrapolated from ^{210}Pb dates). At that time an indifferent (circumneutral) diatom flora dominated, and the planktonic species *Cyclotella kuetzingiana* was very abundant. Diatom-inferred pH was about 6.3 (index B) to 7.1 (WA). Values above 6 (index B) were also calculated for Gårdsjön (Renberg and Hellberg 1982) and Lilla Öresjön (WA) (Renberg et al. 1990) for the preacidification period; these are lakes in southwestern Sweden with a similar recent acidification history. This *Cyclotella*-rich period began 1800 yr B.P. in Lysevatten and 2300 yr B.P. in Lilla Öresjön (Renberg 1990b). It began simultaneously with an expansion of human land use and could be classified as a cultural-induced alkalization and eutrophication period. A similar *Cyclotella* increase was recorded by Engstrom et al. (1985) in Harvey's Lake, Vermont, following European settlement about 200 yr ago and was interpreted to represent increased diatom production caused by accelerated influx of nutrients from agricultural activity in the catchment. The period with higher pH in Lysevatten was preceded by a several thousand year long, more acidic period (Renberg 1990b). However, pH was never below 5 then.

Immediately before the first signs of acidification appear in the sediment record, there is a peak in the net annual accumulation rate of *Cyclotella kuetzingiana* and also *Cyclotella glomerata*, between ca. 1700 and 1900. The same phenomenon was recorded in Gårdsjön and was suggested to be a transitory "eutrophication" period caused by increased fall-out of nitrogen and phosphorus from the atmosphere and inflow of nutrients and base cations from the catchment soils during the initial phase of increased acidity of precipitation before the pH of the lake began to decrease (Renberg et al. 1985). A flush of bases and nutrients from the soils as sulfur deposition from air pollution increased was postulated by Eriksson (1988). Kilham (1982) reported alkalization of a lake in a nonsensitive region in Michigan (USA) as a consequence of acid precipitation over the past 30 yr, primarily due to enhanced rates of carbonate weathering in the catchment caused by a slight lowering of the soil pH.

The first indication of change of the diatom assemblage, of the type usually associated with lake acidification, is dated to ca. 1850 by ^{210}Pb. At the same time, the deposition of carbonaceous particles from fossil fuel combustion began to increase slightly (Fig. 1). This event has been dated to 1850–1900 by analyses of varved lake sediments from other areas in Sweden (Renberg and Wik 1985a) and by ^{210}Pb dating from Lilla Öresjön (Wik and Natkanski 1990). The *Cyclotella* accumulation rate decreased markedly from the late nineteenth century, which largely could explain the composition of the pH category spectra and the diatom-inferred pH curves. However, even if *Cyclo-*

tella is excluded from the diatom sum before calculations of the pH category spectra are made, the trend of acidification from ca. 1850 remains. The first stratigraphic signs of acidification were dated to ca. 1900 in Lilla Öresjön (Renberg et al. 1990).

The beginning of acidification as early as the nineteenth century conflicts with the common view, derived mainly from observations of changing fish populations, of lake acidification in Sweden as being a postwar phenomenon. It is also contrary to the report from 1900 by Trybom (1903) of a rich population of roach in Lysevatten and the diminished population that was still present in the lake in 1948 (Törnquist 1948–49). The paleolimnology and the fishery views might, however, not be conflicting. Both the *Cyclotella* decline and the initial slow increase of acidophilous and acidobiontic diatoms already from 1850 can be explained in terms of acidification-related changes (but not acute acidification causing damage to fish) and by vertical sediment mixing.

The *Cyclotella* decline from about 1 million valves·cm^{-2}·yr^{-1} deposited ca. 1850 to 0.2 million·cm^{-2}·yr^{-1} being deposited 100 yr later could be due to changed chemical composition of the runoff water from the soils around the lake due to progressive soil acidification. It is also important to keep in mind that the picture of massive decline is reinforced by the preceding *Cyclotella glomerata* maximum (Fig. 4) and also that the *Cyclotella* flora appeared in the lake 1800 yr B.P., most likely as a result of human catchment disturbance (Renberg 1990b), and its presence might have been governed also by factors other than pH, such as nutrients.

The percentage increase of acidophilous and acidobiontic taxa from about 1850 is partly due to the decline of the *Cyclotella* species which are classified as indifferent (circumneutral) but does not entirely explain the observed shift between pH categories, as mentioned above. There is, however, another important factor involved, and that is the smoothing of the sediment microfossil records by vertical mixing that occurs in sediments affected by bioturbation and other processes that disturb the sediment deposition. Any level in a mixed sediment is composed of material which is both older and younger than the mean age obtained by dating with radiometric methods. Sudden changes, such as those associated with acidification, e.g. the appearance or expansion of certain acidophilous diatom species, will thus have a date older than the real date of change. For example, a species that appears or flourishes one year will be worked down into older sediment layers, and this apparent age problem increases, of course, with increased mixing depth. To account for this, the faunal changes over time must be considered; the bioturbation was probably larger before acidification than during the acid period because of a decreased abundance of chironomids with acidification (Brodin 1990b).

Acidification proper (with acid episodes and disturbed fish reproduction) in Lysevatten began between ca. 1930 and 1961. The first date is based on the ^{210}Pb dating of the appearance of *Tabellaria binalis*, which is a good acidification indicator in Fennoscandian lakes. This date is probably slightly too old according to the above discussion (and errors in dating must also be considered). In 1961, pH measurements showed that pH fell below 5 in the pelagic zone in both spring and autumn. The fish report by Törnquist from 1948 may indicate disturbed reproduction of roach, which is a species very sensitive to acidification (Hultberg and Stenson 1970; Almer et al. 1974). It is, therefore, most probably that the acidification, in the sense that it caused problems for the fish in the lake, began in the 1940s.

In November 1974 when the lake was limed, the lake water pH had been permanently around 4.5 for a period of 5–10 yr, the lake held no planktonic diatom flora, and periphytic communities were dominated by acidophilous and acidobiontic species. The most striking effect of the liming on the diatom assemblages was the rapid expansion of two indifferent/alkaliphilous periphytic species, *Achnanthes minutissima* agg. and *Cymbella microcephala*, and the planktonic species *Synedra acus*. The liming, however, also affected other species; the trend of a higher abundance of indifferent (circumneutral) taxa is clearly visible even if *Achnanthes minutissima* agg., *Cymbella microcephala*, and *Synedra acus* are excluded from the diatom sum. There was no recovery of the planktonic *Cyclotella* community. The same response to liming was recorded in paleolimnological investigations of two lakes in the United Kingdom (Flower et al. 1990) and has been observed in several limnological studies (Hörnström and Ekström 1986; Larsson 1988; Roberts and Boylen 1989). Hörnström and Ekström (1986) studied a large number of lakes in southwestern Sweden and found diatom plankton recovery in some lakes and stressed the importance of phosphorus as the limiting factor. Experiments with light phosphorus and nitrogen fertilization of limed lakes to some extent confirm this opinion (Olofsson et al. 1988). They concluded that nutrient-starved but still intact plankton communities in lakes under the influence of acid precipitation can be restored as regards the level of authochthonous production, but in severely acidified lakes the obstacle to restoration is the difficulty of recreating the efficient plankton community structure of unacidified lakes (Olofsson et al. 1988). Since, however, diatoms have a remarkable ability to quickly colonize waters (Round 1981), the lack of recovery of the *Cyclotella* flora can hardly be due to the time factor, but more likely to a failure to recreate preacidification water chemistry. Although the pH of the lake is corrected by the liming, the catchment soils and water of Lysevatten are still altered by the acidification. Also, diatoms are well known to respond to other variables besides pH (Birks et al. 1990a).

After the liming in 1976, alkalinity and pH gradually deteriorated and pH leveled out at about 5.0 at the beginning of the 1980s. This reacidification led to decreased abundance of *Achnanthes minutissima* agg. and *Cymbella microcephala* and an increase of acidophilous and acidobiontic diatoms present before the liming. However, an extensive, abnormal bloom of a *Mougeotia* sp. occurred during the reacidification period (Alenäs et al. 1991).

The surficial sediment sample taken in September 1987, almost a year after the second liming, consisted of 80% *Synedra acus* and about 10% was *Achnanthes minutissima* agg. There was still no *Cyclotella* recovery. This *Synedra* dominance also indicates abnormal lake water chemistry.

Paleolimnological results from this investigation and from Renberg (1990b) and all available historical data from Lysevatten and other similar lakes in this area (Hultberg and Andersson 1982; Hultberg 1985) have been compiled to produce a model for the pH development of the lake during the last 2000 yr. The changes during the twentieth century, which are known and reconstructed in the greatest detail, are presented in Fig. 5. The following phases are suggested: (1) 2000 yr ago the lake was naturally rather acid; according to the subfossil diatom record, pH was about 5–5.5; (2) 1800 yr B.P. (calibrated ^{14}C date), pH suddenly increased to above 6 and pH remained at that level until the lake acidified in the twentieth century; a pH maximum occurred between about 1700 and 1900 (diatom-

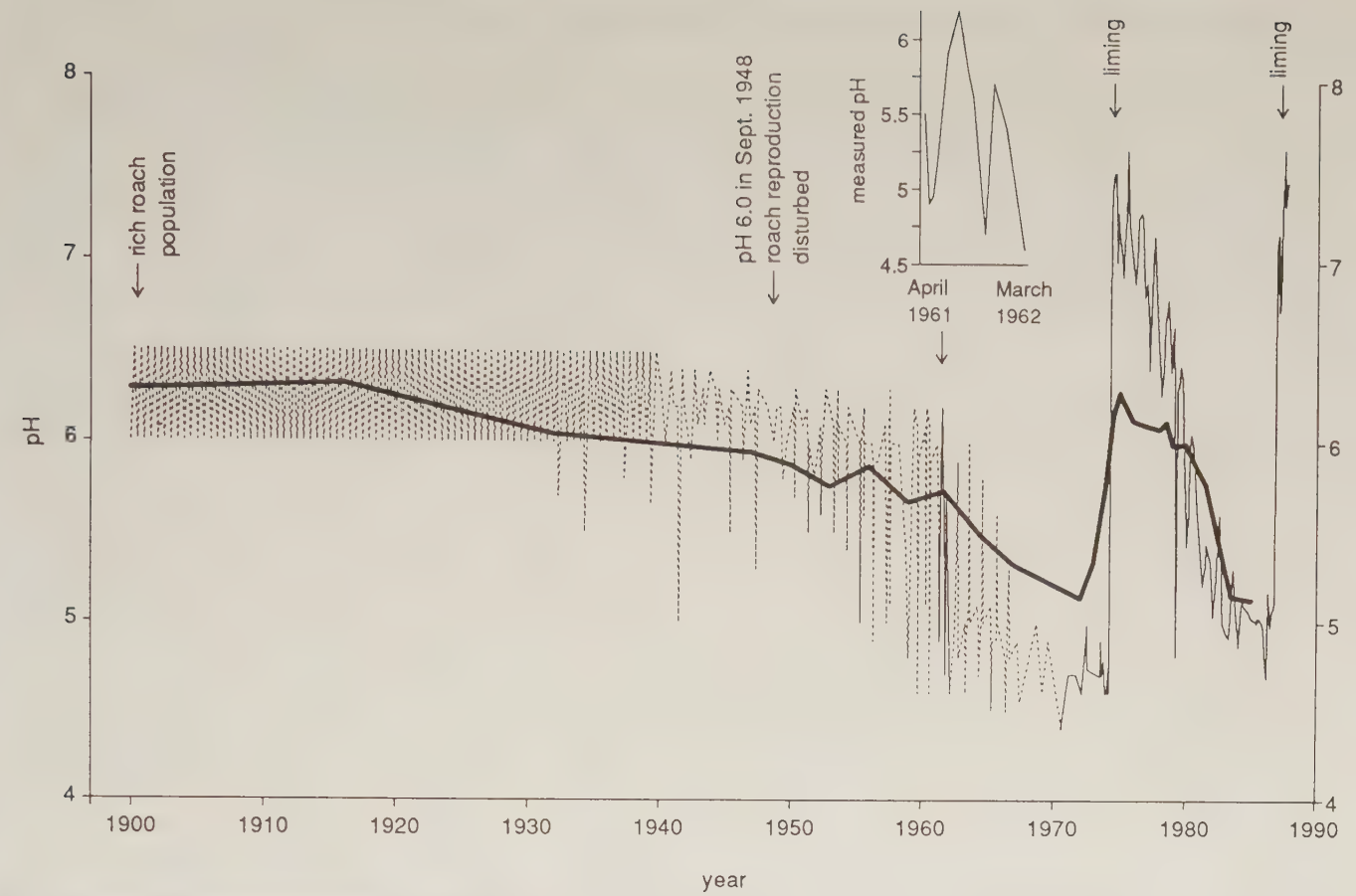

FIG. 5. Reconstruction of the pH history of Lysevatten based on historical data and inference from the subfossil diatoms in the sediment. Historical data are pH measurements (thin solid line) and indirect data from fish reports and data from other similar lakes (thin broken line). The insert, showing pH variations from April 1961 to March 1962, is based on real measurements. Diatom-inferred values (thick solid line) were obtained by weighted averaging.

inferred pH values are about pH 7); (3) rather stable conditions with natural pH variations around 6.0–6.5 occurred between 1900 and 1930, but with changing chemical composition of inflowing water from the catchment and perhaps occasional short acid episodes in the littoral zone in spring; (4) more regular and larger pH drops in spring and autumn, also affecting the pelagic zone, occurred between 1940 and 1960; (5) severe acidification with short recovery periods of pH during summers and winters occurred in the 1960s; (6) permanent acidic conditions (pH 4.4–4.9) occurred from the late 1960s to the liming in April 1974; (7) a rapid increase of pH from 4.5 to 7.5 occurred at liming; (8) pH decreased gradually from 1975 to 1980 when the pH value began to level out at pH 5; and (9) a rapid increase of pH occurred in November 1986 when the lake was limed again. There are now plans for a third liming.

The purpose of the large Swedish liming program (>SEK 100 million·yr^{-1}) is to raise the pH and alkalinity in target waters to above 6 and 0.1 mequiv·L^{-1}, respectively, and to detoxify the waters so that the naturally occurring flora and fauna can persist or recolonize the limed water (Nyberg and Thörnelöf 1988). The program has largely focused on healthy fisheries. However, as this study and others show (Weatherley 1988), liming does not restore the natural, preacidification conditions of the lakes. Monitoring programs and research projects concerning short- and long-term effects of liming are in progress in many countries. In Sweden, these are under the auspice of several authorities (Fleischer et al. 1990). In studies of long-term effects, paleolimnological techniques should be valuable. The large collection of limed lakes in Sweden, some limed as early as the 1950s, provides interesting study material. By analysis of diatoms and other biological remains in sediment cores from lakes of differing type, lakes with varying preliming chemical and biological status, lakes having been limed at different times in the past, and also lakes that have been both limed and fertilized, a better understanding of the ecological consequences of liming could be achieved sooner than solely by traditional monitoring and limnological investigations. This study clearly demonstrates good agreement between sediment and documentary records of acidification and liming in Lysevatten and as such indicates the reliability of the paleolimnological approach.

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Planktivorous Baltic Herring (*Clupea harengus*) Prey Selectively on Reproducing Copepods and Cladocerans

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Flinkman, J., I. Vuorinen, and E. Aro. 1992. Planktivorous Baltic herring (*Clupea harengus*) prey selectively on reproducing copepods and cladocerans. Can. J. Fish. Aquat. Sci. 49: 73–77.

Plankton and Baltic herring (*Clupea harengus*) were sampled simultaneously at nine sites in the northern Baltic Sea and Gulf of Bothnia. The stomachs from 45 herring and 54 plankton samples were analysed. The prey consisted mainly of adult mesozooplankters: copepods and cladocerans. Larger zooplankters (mysids and amphipods) were very rare in the stomachs as were the younger stages (copepodids) of copepods. The stomach contents of the fish changed from brackish water and neritic plankton species in the northern Baltic Proper to limnic species in the northern part of the Bothnian Sea. The diversity of plankton species decreased along with the salinity from south to north. Comparison of the plankton samples and herring stomach contents showed that prey with a large body size was selected as food. Generally this resulted in female copepods being chosen, since they are larger than males of the same species. Especially preferred food items were species and stages which carried conspicuous egg sacs (*Eurytemora affinis* females) or pigmented eggs and embryos (*Bosmina longispina, Podon* spp). Our results suggest that the Baltic herring is capable of exerting a regulative effect on the prey population comparable with that found in freshwater planktivores.

Le plancton et le hareng (*Clupea harengus*) ont fait l'objet de prélèvements simultanés en neuf lieux situés dans le nord de la mer Baltique et le golfe de Bothnie. Les estomacs de 45 harengs et 54 échantillons de plancton ont été analysés. Les proies étaient surtout représentées par des organismes adultes du mésoplancton, à savoir des copépodes et des cladocères. Les plus gros organismes zooplanctoniques (mysidés et amphipodes), de même que les copépodes de stades de développement peu avancés, n'ont été que très rarement retrouvés dans les estomacs. Les estomacs des poissons de la partie nord de la Baltique contenaient surtout des espèces planctoniques des zones néritiques et d'eaux saumâtres tandis que ceux de la partie nord de la mer de Bothnie contenaient surtout des espèces limnétiques. La diversité spécifique du plancton diminuait, comme la salinité, du sud au nord. La comparaison des échantillons de plancton et des teneurs des estomacs des harengs a permis de montrer une préférence pour les proies de grande taille. De façon générale, les copépodes femelles étaient préférés car ils sont plus gros que les copépodes mâles. Les proies les plus recherchées étaient représentées par les espèces présentant, à certains stades de développement, des enveloppes à oeufs très visibles (femelles de *Eurytemora affinis*) ou des oeufs et des embryons pigmentés (*Bosmina longispina, Podon* spp.). Nos résultats portent à croire que le hareng de la Baltique est en mesure d'exercer un effet régulateur sur les populations de ses proies qui est comparable à celui noté chez les planctivores dulcicoles.

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The composition of food consumed by the Baltic herring (*Clupea harengus*) has previously been studied mainly in coastal areas (Popiel 1951; Sjöblom 1961; Aneer 1975, 1980; Ojaveer and Simm 1975; Sandström 1980; Isaksson 1986). Aro et al. (1986), however, studied the composition of food in pelagic environments in summer and reported the importance of smaller zooplankters (copepods and cladocerans) relative to mysids. Previous studies on the feeding of the Baltic herring (review by Sjöblom 1961) have usually identified food only according to relatively high taxonomic groups: genus or order (sometimes even subclass). Hypothesizing that the herring does not feed according to such rough groupings, but is a more sophisticated feeder, we studied the composition of its food at the species level, and even more precisely.

Selective planktivores control the composition and functioning of pelagial lake ecosystems by visual choice of large prey species (for review, see Kerfoot and Sih 1987). This aspect has only recently received attention in the Baltic Sea (Vuorinen et al. 1983; Vuorinen 1987; Rydstam 1988; Hansson et al. 1990). As far as we know, the only earlier study of food selection by Baltic herring is that of Sandström (1980). He showed that the Baltic herring is a selective predator that prefers larger prey species and stages. This suggests that the herring may control the structure and functioning of its prey populations by

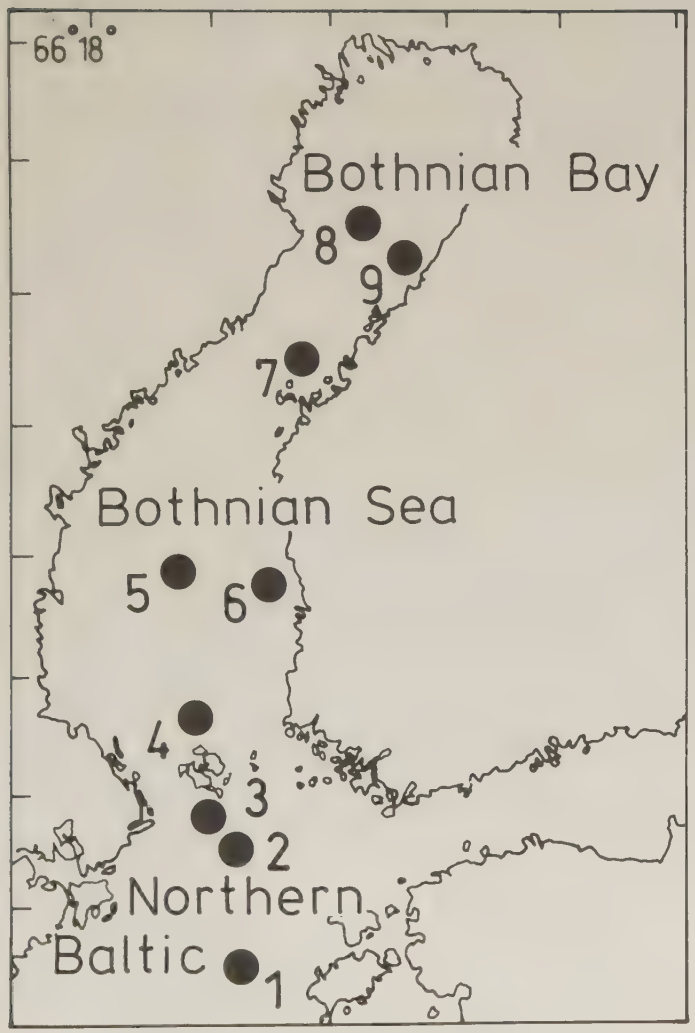

FIG. 1. Trawling and zooplankton sampling localities used in 1985 in the feeding study of the Baltic herring. Stations: 1 = Gotska Sandön, 2 = Bogskär, 3 = Lågskär, 4 = Sälskär, 5 = Eystrasaltbanken, 6 = Mäntyluoto, 7 = Valassaaret, 8 = Ulkokalla, 9 = Falkensgrund.

visual planktivory. Our first working hypothesis was that if the Baltic herring is predominantly a visual planktivore, there should be a positive correlation over large areas between the mean size of prey species and their selection indices. The hypothesis implies that, since female copepods are generally somewhat larger than males of the same species, they are preferred as food. Both particulate and filter feeding have been documented in herring in other areas (Gibson and Ezzi 1985; Batty et al. 1986), but as far as we know, their relative importance has not been studied in the Baltic Sea. In addition to size, pigmentation of eggs increases the visibility of some species and makes them more vulnerable to predation (Mellors 1975). Our second working hypothesis was that egg- or embryo-carrying cladoceran females should be selected more than non-reproducing ones in the herring diet.

Material and Methods

The samples were collected at nine sites during 27 July – 8 August 1985 (Fig. 1). Fish were taken with a Larsen trawl at depths between 4 and 30 m, close to the thermocline (10–25 m), and where fish shoals were detected with an echo sounder. The towing depth and gape of the mouth were observed with a sonde attached to the trawl. The towing speed was three knots for a duration of 1 h. Trawling took place between 23:00 and 00:00, which is the time of dusk in the study area and also the principal feeding time of the Baltic herring (Sjöblom 1961). Immediately after the trawling, the stomach contents of five fish were preserved in formalin. Less than 15 min elapsed between the end of trawling and preservation of the stomachs.

Mesozooplankton samples were taken with a WP-2 net before and after the trawling (three replicates on each occasion). Realising the impossibility of sampling plankton in the same location where the fish had been eating (the fish captured had probably been eating for some time before trawling), we assumed that the mesozooplankton found in the uppermost 25 m of water would represent the food available to the fish. The plankton nets were towed vertically from a depth of 25 m (i.e. from below the thermocline) to the surface at a speed of 0.5 m/s. The plankton samples were preserved in formalin.

Microscope slides were prepared from the stomachs in order to identify and count smaller zooplankton. In a preliminary study, successive groups of 20 zooplankton individuals were identified on five slides until the variation in the proportional abundance of different species levelled off (Elliot 1971). The preliminary study showed that counting 100 individuals on each slide gave a good estimate of the relative abundances of different species. The WP-2 plankton samples were counted in a laboratory of the Finnish Institute of Marine Research using the standard methods for Baltic zooplankton (Anonymous 1984). In counting the samples, plankton was identified according to species; copepods were classed according to developmental stage (adult and copepodid) and sex. In counting cladocerans, ovigerous and nonovigerous females were recorded separately. The identification categories are shown in Table 1. The plankton and stomach samples were compared using the average abundance percentages for each identification category in the stomach samples and the averages in the plankton samples for each sampling station. Size-based selection was evaluated by ranking the zooplankton species according to average species-specific biomass values (Hernroth 1985) (Table 1). Selectivity was studied with the *V*-index (Pearre 1982) which is based on the chi-square test:

$$V = \pm(\chi^2/n)^{1/2}.$$

The *V*-index values for reproducing (ovigerous) and non-reproducing cladocerans were compared using the Mann–Whitney *U*-test (Sokal and Rohlf 1981).

Results

On the basis of the species composition in the plankton and stomachs, the study area can be divided into three subareas: northern Baltic Proper (sampling stations 1–3), Bothnian Sea (stations 4–6), and Bothnian Bay (stations 7–9). The main difference between the areas is the occurrence of relatively large neritic copepods, *Temora, Centropages*, and *Pseudocalanus*, in the northern Baltic Proper and their absence from the Bothnian Sea and Bothnian Bay (Fig. 2). In the Bothnian Bay, a freshwater species, *Limnocalanus macrurus*, is the dominant copepod. Medium-sized mesozooplankton species are found throughout the study area, the copepods *Eurytemora affinis* and *Acartia bifilosa* being the most important. Abundant cladocer-

TABLE 1. Identification categories and size (Hernroth 1985) and size ranking of mesozooplankton studied in the northern Baltic Sea in summer 1985. Ovofemales refer to egg- or embryo-carrying cladoceran females and have the same size as females without eggs or embryos.

Rank	Identification category	Average size (μg)
1	*Acartia bifilosa* copepodids	12
2	*Eurytemora affinis* copepodids	14
3	*Bosmina longispina* females	15
4	*Bosmina longispina* ovofemales	15
5	*Temora longicornis* copepodids	15
6	*Centropages hamatus* copepodids	15
7	*Acartia bifilosa* males	17.5
8	*Pseudocalanus elongatus* copepodids	20
9	*Eurytemora affinis* males	20
10	*Evadne nordmanni* females	20
11	*Evadne nordmanni* ovofemales	20
12	*Acartia bifilosa* females	22.5
13	*Podon polyphemoides* females	25
14	*Podon polyphemoides* ovofemales	25
15	*Eurytemora affinis* females	30
16	*Pseudocalanus elongatus* males	35
17	*Centropages hamatus* males	40
18	*Temora longicornis* males	45
19	*Centropages hamatus* females	45
20	*Pseudocalanus elongatus* females	50
21	*Limnocalanus macrurus* copepodids	50
22	*Temora longicornis* females	60
23	*Podon intermedius* females	200
24	*Podon intermedius* ovofemales	200
25	*Limnocalanus macrurus* males	454
26	*Limnocalanus macrurus* females	810

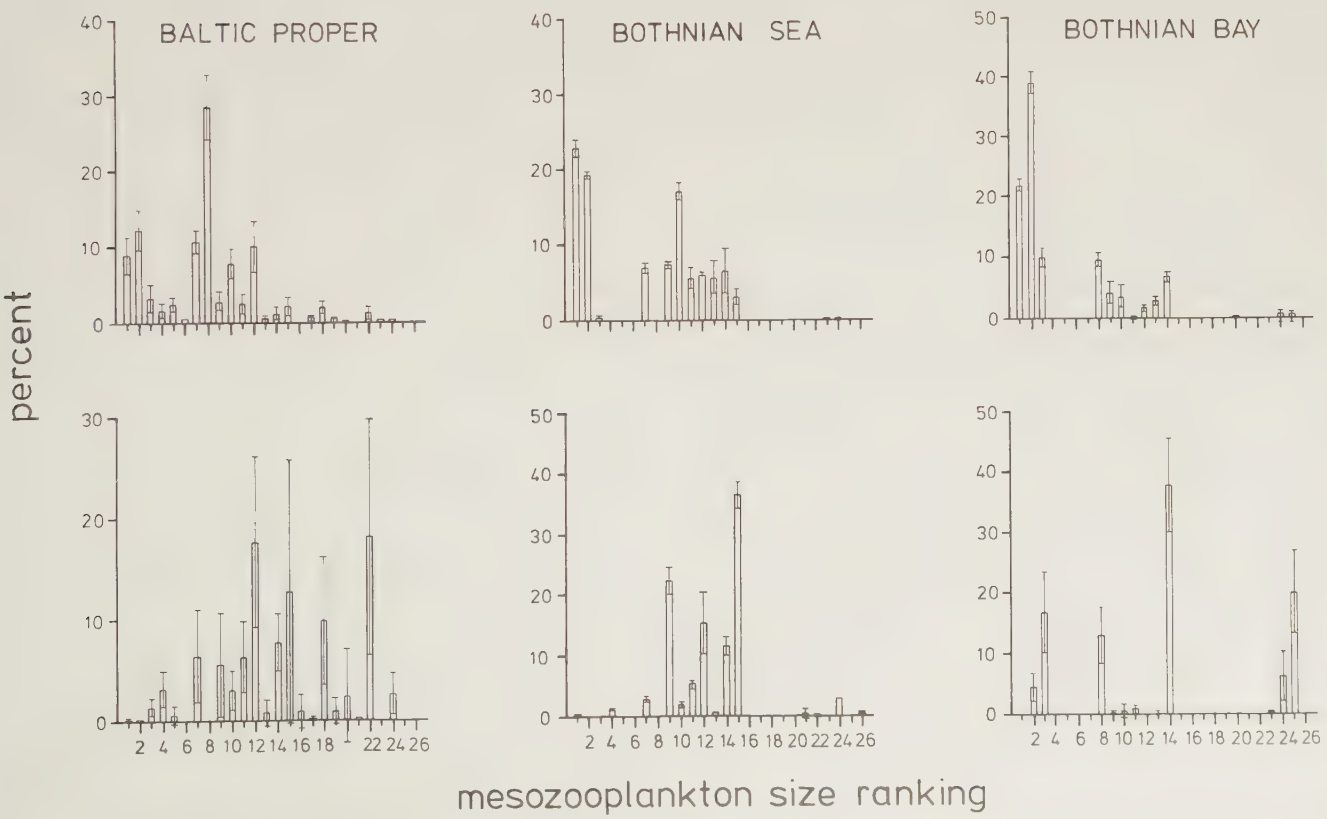

FIG. 2. Percentage of different size groups in mesozooplankton samples (upper panels) and stomach contents of Baltic herring (lower panels) collected simultaneously in the northern Baltic Sea in summer 1985. Plankton $n = 6$ tows at each location; fish $n = 5$ in each location (except for Valassaaret, where $n = 2$), vertical bars = standard deviation. Size ranking categories refer to identification categories listed in Table 1.

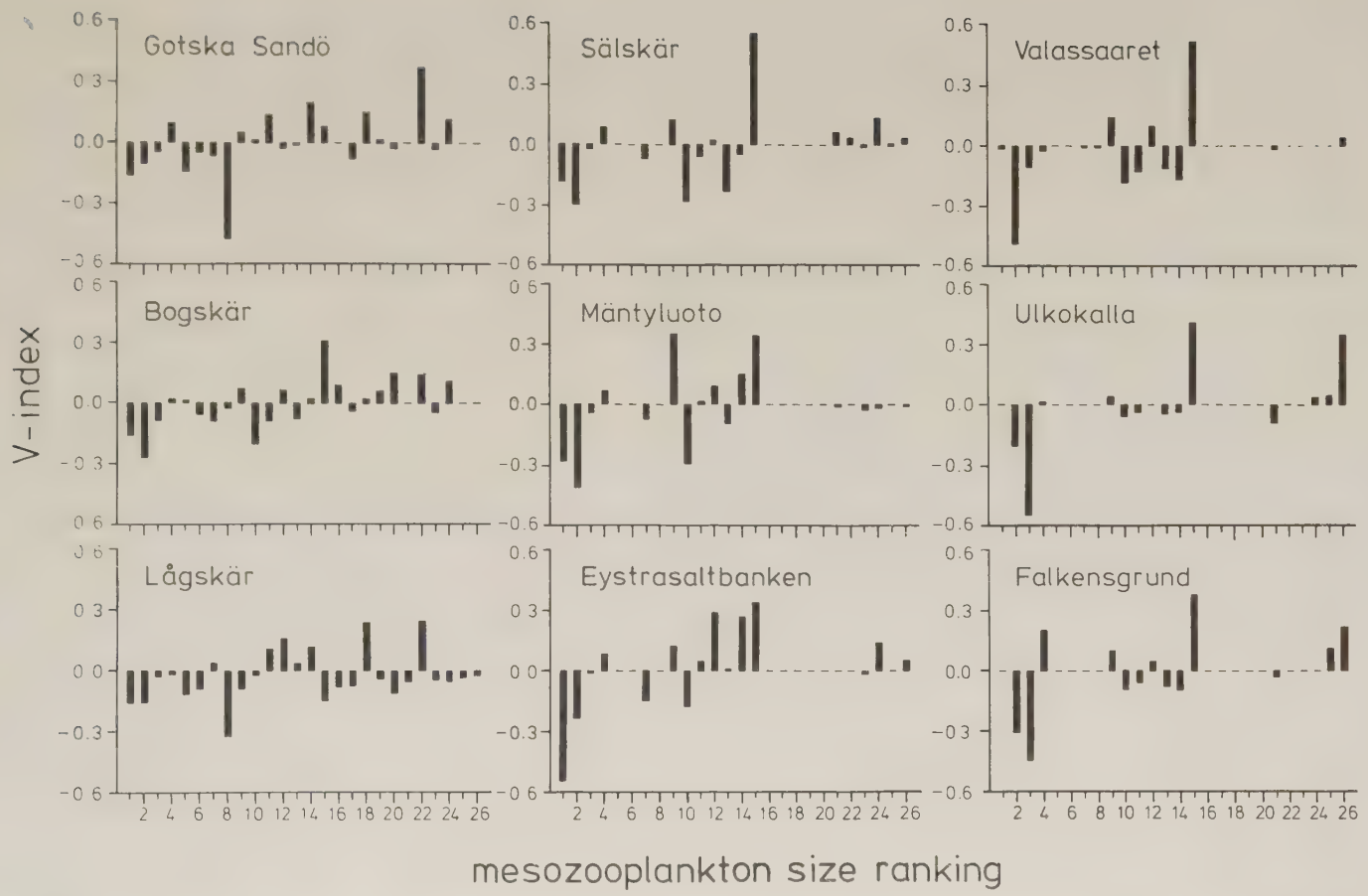

FIG. 3. Food selection indices (Pearre 1982) for different-sized zooplanktonic prey species of the Baltic herring in the northern Baltic Sea in summer 1985. Size ranking categories refer to identification categories listed in Table 1.

ans are *Podon polyphemoides, Evadne nordmanni*, and, especially, *Bosmina longispina*. The food composition of the Baltic herring reflected a change from neritic species in the northern Baltic Proper to brackish water and limnic species in the Bothnia Sea and Bothnian Bay.

There were marked differences between stomach contents and plankton samples in the abundance percentages of different species. Our first hypothesis was supported, since the size of items found in the stomachs of herring was, in general, larger than in the plankton samples (Spearman correlation coefficient $r_S = 0.332$, $p < 0.001$, between V-index and size rank; Fig. 3). A corollary of the hypothesis was that copepod females are preferred over males of the same species. This was also corroborated, since in 29 of 34 stomachs the V-index values of the females were higher than those of males of the same species (Fig. 3). Although it is not the largest zooplankter in the area, *Eurytemora* is generally the most important prey species in the whole study area. It seems to be selected more frequently than expected on the basis of size alone (Fig. 2). Among cladoceran species that carry eggs or embryos, e.g. *Podon* species and *Bosmina*, the progeny-carrying females are represented more in the diet than those without, and their selection index values are higher than those of nonovigerous females (Table 2; Fig. 3); this supports our second hypothesis.

Discussion

In all the areas studied the Baltic herring showed a preference for larger prey species. In consequence, neritic copepods (*Temora, Centropages*) were the preferred food in the northern Baltic Proper, while large limnic species (*Limnocalanus*) or ovigerous stages of smaller copepods (*Eurytemora*) were the principal food item in the more limnic Bothnian Bay. The females of all copepod species in this study are larger than the males of the same species and they are the target of selective predation by herring, which can be expected to decrease the reproductive success of the species. In the northern Baltic Proper, the medium-sized *Eurytemora* is probably protected from predation to some extent by the abundance of larger neritic copepods; it is not selected as heavily in that area as in the Bothnian Sea and Bothnian Bay.

Body size, however, is not the only criterion for prey selection. For example, ovigerous *Podon intermedius* females were often found in the stomachs although they are very rare in the plankton samples (Fig. 3). Furthermore, reproducing, egg-carrying females of the two *Podon* species and *Bosmina longispina* are selected as food more than those without eggs (Table 2). The presence of pigmented eggs probably makes these plankters conspicuous and more likely to be selected as prey.

Direction of predation towards reproducing individuals would effectively prevent a zooplankton species from achieving the maximum population growth rate (e.g. *Eurytemora*, see Vuorinen 1987). We hypothesize that the Baltic herring may control the formation and functioning of the pelagic zooplankton community. If this is true, the regulating effect should first be visible in large neritic copepods in the Baltic Proper, while

TABLE 2. Prey selectivity values (*V*-index, Pearre 1982) of Baltic herring for cladocerans with (A) or without (B) eggs or embryos in the northern Baltic Proper, Bothnian Sea, and Bothnian Bay in summer 1985. *U* = Mann–Whitney test statistic for differences between A and B (NS = not significant).

	Podon intermedius		*Podon polyphemoides*		*Evadne nordmanni*		*Bosmina longispina*	
Station	A	B	A	B	A	B	A	B
Northern Baltic								
1	0.12	−0.03	0.2	−0.01	0.14	−0.01	0.1	−0.05
2	0.11	−0.05	0.02	−0.08	−0.9	−0.2	0.02	−0.09
3	−0.05	−0.04	0.12	0.4	0.11	−0.02	−0.02	−0.03
Bothnian Sea								
4	0.13	−0.02	−0.05	−0.24	−0.7	−0.29	0.08	−0.03
5	0.14	−0.02	0.27	0.01	0.5	−0.18	0.08	−0.01
6	−0.02	−0.03	0.15	−0.1	0.01	−0.3	0.06	−0.05
Bothnian Bay								
7	0.0	0.0	−0.17	−0.12	−0.13	−0.19	−0.03	−0.11
8	0.0	0.0	−0.1	−0.08	−0.6	−0.1	0.2	−0.45
9	0.04	0.0	−0.04	−0.05	−0.4	−0.6	0.01	−0.55
U	40.5		55.0		43.0		78.0	
p	0.018		0.200 NS		0.825 NS		0.001	

in the Bothnian Sea and Bothnian Bay, where neritic copepods are not common, *Eurytemora, Podon*, and *Bosmina* should be the main species controlled by the Baltic herring.

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Population Structure of Female and Immature Sperm Whales (*Physeter macrocephalus*) off the Galápagos Islands

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Whitehead, H., S. Waters, and T. Lyrholm. 1992. Population structure of female and immature sperm whales (*Physeter macrocephalus*) off the Galápagos Islands. Can. J. Fish. Aquat. Sci. 49: 78–84.

The structure of the population of female and immature sperm whales (*Physeter macrocephalus*) in the region of the Galápagos Islands was studied using individual photographic identifications of 1285 animals collected between 1985 and 1989. Population parameters were estimated using a maximum-likelihood mark–recapture estimate permitting emigration from the study area in which identifications are collected and then reimmigration back into it. Because permanent associations among whales violated assumptions of independence, confidence intervals for the estimates were constructed using Monte-Carlo population simulation. The analysis suggested that there is a population of very approximately 200 whales in the study area around the islands at any time. These were part of a larger population numbering between 2600 and 5300 individuals (95% confidence interval). An average of 39–94% (95% confidence interval) of the whales left the study area in any month, with a similar number immigrating.

Les auteurs ont étudié la structure de la population de cachalots (*Physeter macrocephalus*) femelles et immatures des environs des îles Galápagos à l'aide de photographies d'identification individuelle de 1 285 animaux obtenues de 1985 à 1989. Les paramètres de population ont été déterminés par une estimation par maximum de vraisemblance des marquages et recaptures qui permettait de tenir compte de l'émigration hors de la zone d'étude où les identifications avaient été faites et du retour des individus. Comme l'existence d'associations permanentes entre des cachalots invalide les hypothèses d'indépendance, les intervalles de confiance des estimations ont été établis par simulation Monte-Carlo de la population. L'analyse indique que la population permanente de cachalots de la zone d'étude s'élevait, très approximativement, à 200 individus dans les environs des îles. Ces cachalots faisaient partie d'une population plus importante dont l'effectif se situait entre 2 600 et 5 300 individus (intervalle de confiance de 95 %). Au cours de chaque mois, de 39 à 94 % environ (intervalle de confiance de 95 %) des cachalots quittaient la zone d'étude, mais un nombre équivalent d'individus y pénétraient.

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The geographical structure of sperm whale (*Physeter macrocephalus*) stocks has been of particular interest to the Scientific Committee of the International Whaling Commission (e.g. International Whaling Commission 1980) and others concerned with sperm whale population dynamics. Much of our ability to predict the effects of exploitation or protection on sperm whales in a particular ocean depends on an accurate assessment of the structure of their stocks. In recent years, attention has focused on the division of male sperm whale stocks in the North Pacific (International Whaling Commission 1987). Evidence for and against different proposed stock divisions has been put forward using sighting and catch distributions, returns of artificial marks by the whaling industry, and genetic analyses (e.g. International Whaling Commission 1980).

There has been much less work on stock identity in female sperm whales, although Best (1979) compared distances covered by animals that were artificially marked and later killed, finding that females seem to have generally smaller ranges than males. Off the Galápagos Islands, Ecuador, we have been collecting photographic identifications of individual sperm whales, principally females, since 1985, and these have allowed us to begin to examine stock structure (Whitehead and Waters 1990).

Our first analyses of the data suggested that the females and immatures identified off the Galápagos could be clustered into approximately closed "groups" containing about 20 animals each (Whitehead and Arnbom 1987; Whitehead and Waters 1990). Groups seemed to migrate into and out of our study area near the Islands, and there was some indication that there might be reimmigration, with groups moving away from the Islands to later return (Whitehead and Waters 1990). This possible reimmigration invalidates most of the mark–recapture techniques which are used to examine population size and other parameters from individual identifications (Hammond 1986; Seber 1982). However, Whitehead (1990a) has developed a maximum-likelihood mark–recapture technique which does permit reimmigration and, by estimating the rate of reimmigration, can provide important information on stock structure. Use of this technique on the identification data collected off the Galápagos in 1985 and 1987 gave a rough population estimate for the study area around the Islands where the data were collected and demonstrated the presence of immigration into and

out of the area. However, it was unable to differentiate between cases of substantial reimmigration, representing a small total population, and no reimmigration, suggesting a very large total population (Whitehead and Waters 1990).

Additional identification data were collected during field seasons in 1988 and 1989. With these data we have been able to examine the social organization of the female and immature sperm whales in greater detail (Whitehead et al. 1991). Each individual was found to be a member of a permanent "unit" consisting of approximately 13 whales. These permanent units associated with one another for periods of approximately 6.5 d. An average of about two of these units were together at any time, forming the groupings of roughly 20 individuals noted in the earlier studies.

This more elaborate social structure has necessitated some changes in the statistical techniques used to examine population structure. We had originally carried out the population analysis on groups of identified whales constructed using the temporal patterns of identifications within days (Whitehead and Arnbom 1987; Whitehead and Waters 1990). The discovery that these groups generally consist of temporary (over periods of a few days) associations of units invalidated this approach. The associations between units for periods of days also meant that we could only recognize identified whales as members of the same unit if they were photographed together on two or more occasions at least a month or so apart, and thus only a small part of the identified population could be assigned to a unit.

In this paper we present an analysis of the population structure of the Galápagos sperm whales using a version of Whitehead's (1990a) mark–recapture technique, modified to take into account the new results on social structure. The addition of the data collected in 1988 and 1989 also allows us to be more precise about our parameter estimates and, in particular, to assess the size of the parent stock of which the Galápagos whales are a part.

Methods

Field Methods

Identification photographs of female and immature sperm whales were taken during four field studies in 1985 (H. Whitehead), 1987 (H. Whitehead and S. Waters), 1988 (S. Waters), 1988–89 (T. Lyrholm), and 1989 (H. Whitehead) (Table 1). While in the Galápagos, we spent 5- to 14-d periods at sea on board auxiliary sailing vessels (13 m l.o.a. for 1988, 20 m l.o.a. for 1988–89, and 10 m l.o.a for other studies) separated by a few days in port. Virtually all research was carried out within 100 km north, northwest, west, and southwest of the major Galápagos Islands (Whitehead et al. 1989). This region will be referred to as the "study area." We located and tracked groups of sperm whales acoustically for periods of days (Whitehead and Gordon 1986). During daylight, we approached sperm whales visible at the surface as discreetly as possible in order to photograph their flukes as they dived.

Identification

Sperm whales can be identified individually from good photographs of their flukes (Arnbom 1987). Fluke photographs of adult females and immatures greater than 1 yr old, from all studies, were identified and catalogued by S. Waters, with assistance from a computer cataloguing routine (Whitehead 1990b). Each photographic identification was assigned a quality grade from 1 (poor) to 5 (excellent) depending on how well an individual could be recognized with certainty from the identification (Arnbom 1987). A few errors in previous identification lists for 1985 and 1987 were discovered and corrected, and a number of quality grades were reassigned. The numbers of high-quality fluke identifications obtained each year are given in Table 1. There were a total of 3434 identifications of 1285 different whales.

Population Size and Structure

A modified maximum-likelihood mark–recapture method (Whitehead 1990a) was used to examine the size and geographical structure of the population of female and immature sperm whales off the Galápagos, using calendar months as time units. For a given set of population parameters, the likelihood of the observed identification histories of all observed individuals is calculated recursively, and then an optimizing routine is used to find the parameters which maximize this likelihood. The important assumptions of Whitehead's (1990a) method, when applied to the Galápagos population of female sperm whales, are as follows.

(1) In any month, all members of the population within the study area around the Galápagos Islands had an equal probability of being identified.

(2) All members of the population within the study area had the same probability (λ/month) of emigrating out of it.

(3) All members of the population outside the study area had the same probability (μ/month) of immigrating into it.

(4) The probability of identifying a member of the population was independent of whether other members of the population were identified, and the probability of migrating to or from the study area was independent of the migrations of others.

(5) Identification did not affect the probability that a member of the population was reidentified in another month, or that it migrated either to or from the study area.

(6) No misidentifications were made.

(7) The larger population was closed, with no birth and no death, and was of size Z.

TABLE 1. Summary of identifications of female and immature sperm whales collected off the Galápagos Islands between 1985 and 1989.

	Study				
	1985	1987	1988	1988–89	1989
Dates of identifications	23 Feb. – 20 Apr.	3 Jan. – 28 June	12 Apr. – 20 Apr.	11 Nov. – 3 Apr.	13 Apr. – 21 May
Identifications >grade 3	652	1100	232	601	849
Individuals identified	298	379	99	344	325

(8) The population in the study area (N) was reasonably constant in size ($N = Z \cdot (\lambda + \mu)/\mu$). This follows from assumptions 2 and 3.

Estimates of Z, λ, and μ were found using maximum-likelihood methods (Whitehead 1990a), and from these an estimate of N was calculated.

Unlike the previous analyses (Whitehead and Waters 1990) in which groups were used, the raw data for this analysis were the calendar months in which identified individuals were seen. However, as individuals were members of permanent units travelling together, assumption 4, independence, is seriously violated. In many statistical analyses, nonindependence of samples does not bias parameter estimates but it does invalidate hypothesis tests and causes substantial inaccuracies in standard errors and confidence intervals. We therefore provisionally accepted the mark–recapture maximum-likelihood parameter estimates and used a Monte-Carlo simulation (which incorporates the social structure of the whales) to construct approximate confidence intervals for the parameter estimates, as well as to check for bias. Details of this simulation are given in the Appendix (Simulation 1).

The hypothesis that there was no reimmigration (i.e. members did not leave the population to later return, $\mu = 0$, $Z = \infty$) was examined using a similar modification to the likelihood ratio test. As in Whitehead (1990a), the test statistic was the difference between the maximum log-likelihood for a three-parameter model (N, λ, and μ) and the maximum log-likelihood for a two-parameter model (N and λ, $\mu = 0$). The value of this statistic was compared with a distribution of values calculated from simulated populations with no reimmigration ($\mu = 0$) as described in the appendix (Simulation 2).

To test some of the assumptions of the model, characteristics of the identification histories of the observed individuals were compared with those from simulated populations (Appendix: Simulation 3), and alterations were made to the rules governing the simulations (incorporating variability in probability of being identified, or changes in markings (Appendix: Simulations 4 and 5)).

As most sample parameter estimates showed strongly skewed distributions, we generally present medians rather than means and ranges rather than standard deviations. Both the population simulation, and especially the iterative finding of maximum-likelihood population estimates which have strong correlations (Table 3), used considerable computer time. Therefore the number of runs carried out for each simulation varied depending on its purpose and demands on computer time.

Results

Some individual sperm whales were identified in each pair of years except 1985/1988. Many fewer individuals were identified in 1988 than the other years (Table 1). The number of between-year matches, together with the substantial numbers of individuals identified in more than 1 mo during a year, gave sufficient data for the results of the maximum-likelihood mark–recapture method to have some apparent value.

The maximum-likelihood mark–recapture method gave an estimate of very approximately 185 individuals in the study area around the Galápagos Islands at any time with a high turnover (estimated to be about 85% per month) to and from other parts of the range of the parent population which contained an estimated 2600–5300 whales (Table 2). Probably because of the high rate of migration towards and away from the islands, our estimate of the total population is considerably more precise than that of the number of whales in the study area. There are substantial correlations between some of the pairs of parameters, especially N, λ, and μ (Table 3).

The difference between the maximum log-likelihoods for two- (N and λ, $\mu = 0$) and three-parameter (N, λ, and μ) models was 24.89. For 100 simulated populations (Appendix: Simulation 2) using as input the maximum-likelihood estimates from the two-parameter model when applied to the original data ($\mu = 0$, $N = 1985$, $\lambda = 0.0247$), this difference ranged between 0.000 and 11.20 with a mean value of 0.88. Therefore, we reject the hypothesis that $\mu = 0$ at $P < 0.01$ and conclude that individuals, having emigrated from the study area around the Galápagos Islands, had a finite probability of reimmigrating. The results of this test are in contrast with an analysis of only the 1985 and 1987 data in which $\mu = 0$ was not rejected (Whitehead and Waters 1990). The larger data set, collected over a longer period, has greatly increased the power of the test.

Discussion

Validity of Model

Like any other model of a biological system, the model of sperm whale populations used in this paper is unrealistic. Because conclusions may not be valid if the assumptions, as listed above in the Methods section, do not hold, these assumptions, and the effects of possible failures in them, should be scrutinized carefully.

1. Equal identifiability

It is quite possible that some individuals or whole units may have been more identifiable than others, either because of differences in behaviour or because their fluke patterns were more or less identifiable from photographs of a particular quality. We tried to minimize the heterogeneity of identifiability due to different types of marks on different individuals by only using high-quality photographs from which virtually any whale could be identified, and our subjective impression in the field was that most whales behaved similarly. The distributions of the number of months in which each individual was identified were very similar for the actual and simulated (Appendix: Simulation 3) data sets (Table 4), arguing against much differential identifiability. However, some differences in identifiability between individuals, or units, may remain. To investigate the effects of such differences, the population was simulated including variability in the probability that individuals/units were identified, g (Appendix: Simulation 4). The results of these simulations in which g varied randomly between 0.285 and 0.885 are summarized in Table 5. There is no indication that these quite substantial deviations from the assumption of equal identifiability make any important difference to the parameter estimates.

2 & 3. Equal emigration and immigration rates

If some units were more or less likely to emigrate from the study area, then this would skew the distribution of consecutive months in which groups were identified, with more individuals being seen in several consecutive months than expected. If anything, the reverse was true when observed and simulated (Appendix: Simulation 3) data were compared (Table 4). It is harder to test immigration rates, although severe differences in immigration rates would tend to skew the distribution of the number of months in which members of the population were

TABLE 2. Estimated population parameters for Galápagos sperm whales, compared with those derived from 100 simulated (Appendix: Simulation 1) runs of population using estimated parameters.

	Estimate	Simulation	
		Median	95% confidence interval
Total population, Z	3891	4110	2600–5300
Population size off Galápagos, N	185	161	60–890
Emigration from Galápagos area, λ	0.842/mo	0.869	0.39–0.94
Immigration into Galápagos area, μ	0.042/mo	0.037	0.020–0.106
Log-likelihood	−1239.6	−1425.0	−1 000 – −1790

TABLE 3. Correlations between parameter estimates from 100 simulated runs (Appendix: Simulation 1).

	Z	N	λ
Total population size, Z			
Population in study area, N	0.330		
Emigration rate, λ	−0.385	−0.923	
Reimmigration rate, μ	−0.002	0.772	−0.734

identified, as some groups would repeatedly enter the study area and be identified. There is a slight, but far from significant, tendency in this direction (Table 4).

4. Independence

The medians of the population parameter estimates from the 100 simulated runs are close to the original input parameters (Table 2). This suggests that the maximum-likelihood mark–recapture estimates are approximately unbiased, even though the assumption of independence is violated because whales travel in permanent units. The simulation procedure used to calculate confidence intervals incorporates the social structure of the whales and so does not assume independence.

5. Effects of identification

Whales rarely showed any short-term reaction to the presence of our research vessel. Therefore, it seems most unlikely that our identification of an individual affected its likelihood of reidentification in another month or its migrations.

6. Misidentifications

As our data set contains over 3000 photographs, it is likely that there are a few misidentifications remaining in it. It is also possible that some individuals changed their marks sufficiently to be unrecognizable during the study period. We investigated the effects of these types of misidentification using simulated populations (Appendix: Simulation 5) in which individual whales changed their marks to become unrecognizable at rates of $\beta = 0.005$ per month (=5.8% of the population changing their marks per year). The results suggest, as might be expected, that such rates of misidentification may bias Z, the total population size, upwards (by perhaps 10%) and μ, the reimmigration rate, downwards (Table 5). Over periods of 4 yr, we have seen only very small changes in a few of the patterns on flukes that we have reidentified, so we believe that changes sufficient to cause misidentification are very rare. We also have checked our identifications several times and so it seems unlikely that misidentification rates of 5.8% per year are present in the data set. Thus, while misidentification may contribute bias to Z and μ, in our case we believe these biases to be very small compared with the uncertainty in our estimates.

TABLE 4. Numbers of months, and numbers of consecutive months, in which individuals were identified in actual data set and in 500 simulated data sets (Appendix: Simulation 3).

	No. of individuals	
	Observed	Simulated (median (range))
No. of months identified		
1	582	611 (509–702)
2	67	65 (27–119)
3	9	5 (0–24)
4	4	0 (0–7)
5	0	0 (0–3)
No. of consecutive months identified		
1	733	719 (651–759)
2	13	19 (0–54)
3	0	0 (0–10)
4	0	0 (0–5)

7. Closure of population

Whales die, and others are recruited into the identifiable population (at about 1.5 yr of age, as first-year calves rarely show their flukes). They may also immigrate into our total study population, or emigrate out of it. The mortality rate of female sperm whales has been estimated to be 0.055 per year (International Whaling Commission 1987), and the current recruitment rate of the Galápagos population appears to be similar or, possibly, somewhat lower than this (Waters and Whitehead 1990). When recruitment and mortality of approximately equal magnitudes are introduced into a previously closed population, the effect on the distribution of identification histories is similar to that produced by unrecognizable changes in the identification pattern (as a dead whale being replaced by a recruited one is equivalent to a whale changing its marks). Therefore, referring to the results on the simulated populations described above under assumption 6, mortality and recruitment of about 0.055 per year may bias estimates of Z, the total population size, upwards by about 10% and μ, the reimmigration rate, downwards. The external population feeding the Galápagos study area may not be completely closed, but if there was substantial migration between it and other populations, then these would be, at least partially, included in the total population estimate (Z).

8. Variation in population within study area

Both our observations between 1985 and 1989 and an investigation of sighting rates by American whalers working in the

TABLE 5. Summary of results of estimates of parameters of populations simulated using the maximum-likelihood parameter estimates from the data.

	No. of runs	Median and range			
		Z	N	λ/mo	μ/mo
Input (from data)		3891	185	0.842	0.042
Original	100	4110	161	0.869	0.037
(Simulation 1)		2396–6144	63–1963	0.095–0.946	0.020–0.174
Variable unit identifiability	11	3997	250	0.780	0.046
(Simulation 4)		2658–4968	72–615	0.413–0.935	0.019–0.083
Variable individual identifiability	11	3961	157	0.864	0.044
(Simulation 4)		3159–4520	87–503	0.535–0.941	0.019–0.077
Change in pattern 0.005/mo	50	4623	190	0.859	0.033
(Simulation 5)		1755–7539	35–987	0.215–0.962	0.016–0.121

area between 1830 and 1850 suggest only little consistent seasonal variation in the numbers of sperm whales near the Galápagos Islands (Hope and Whitehead 1991; Whitehead et al. 1989). The parameter estimates do not seem to be especially sensitive to the assumption of a constant population in the study area. During simulated runs (Appendix: Simulation 1) using the parameter estimates from the original data, the population in the study area routinely varied between 60 and 500.

In general, then, the information available suggests that the mark–recapture model used in this analysis at least roughly approximates reality, and so may give useful parameter estimates. As indicated by maximum log-likelihood values (Table 2), the model fit the real data slightly better than most of the simulated data. However, the fact that a model fits the data well does not necessarily imply that it is a good model — another, simpler model might fit equally well. But, in our case, the model fitted has only three parameters (Z, λ, and μ) and the rejection of the hypothesis that $\mu = 0$ showed that no restricted model fit as well. Other reasonable models, perhaps based on diffusion principles, might fit our data usefully, but it is hard to imagine such a model with three or fewer parameters.

Signficance of Results

Our results suggest that the female and immature sperm whales found near the Galápagos Islands are part of a larger poulation of 2600–5300 individuals. This suggests that female sperm whales, which in the Pacific are thought to number in the hundreds of thousands (International Whaling Commission 1983), may form reasonably small stocks. Neither the geographical ranges nor the degree of separation of these stocks are presently known, but useful information might come from genetic analyses, or individual identification studies in other areas.

Analysis of logbooks of American whaling voyages between 1830 and 1850 suggested a population near the start of exploitation of very roughly 10 000 sperm whales in a 10° latitude by 10° longitude area centred on the Galápagos Islands (Hope and Whitehead 1991). A comparison with the estimate for the current total Galápagos population derived in this paper might suggest that either the population is considerably reduced from previously or that it has a range less extensive than the 10° square. However, both estimates suffer from considerable uncertainty so that such comparisons must be made very cautiously.

With perhaps one third of the estimated population identified, additional photographic identification studies in areas near the Galápagos should help determine the home range of the Galápagos population and the degree to which it mixes with other stocks.

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Appendix: Population Simulation

In order to obtain confidence intervals for the maximum-likelihood estimates of the population parameters, as well as to check for bias in the estimation, the population of female sperm whales was simulated using Monte-Carlo methods. The following parameters were used: Z = total population size (from maximum-likelihood estimate); λ = emigration rate (from maximum-likelihood estimate); μ = reimmigration rate (from maximum-likelihood estimate); U = mean size of permanent units (= 13 whales, from Whitehead et al. 1991); W = mean number of whales identified each calendar month in which field research was carried out (=93.4 whales); g = probability that an individual was identified in any month given that its unit was identified that month. Individuals were allocated to units if they were identified within 2 h of one another on two occasions at least 30 d apart (Whitehead et al. 1991). g was estimated from

$$g = \sum_u \sum_m \sum_i M(u, m, i) \Big/ \sum_u \sum_m \sum_i P(u, m, i)$$

where $P(u, m, i) = 1$ for individual i of unit u in month m if some member of the unit other than i was identified that month and i was identified during at least two other months, and $P(u, m, i) = 0$ otherwise. $M(u, m, i) = 1$ if $P(u, m, i) = 1$ and i was identified in month m, and $M(u, m, i) = 0$ otherwise. g was estimated to be 0.585.

Simulation 1

Z/U units were created, each with q individually known whales where q was uniformly distributed between 1 and $2U + 1$. This distribution was suggested by the variation in the sizes of the units created by the "seen together 30 or more d apart" criterion. Initially, each created unit was placed within the Galápagos study area with probability $\mu/(\lambda + \mu)$. Between each pair of consecutive months, units within the Galápagos study area moved out with probability λ and units outside moved in with probability μ. This created a pool of units, each with its individually identified whales, in the study area each month.

If, in month m (in which there had been field research), this pool was of size $B(m)$ whales, then the number of whales to be identified that month was $I(m) = B(m) \cdot W/N$ ($N = Z \cdot \mu/(\lambda + \mu)$). We could not use the actual number of identifications each month because, in some runs for some months, $B(m)$ was smaller than this. For each month in which field research had been carried out, units within the Galápagos pool were selected sequentially and randomly without replacement, and each member of a selected unit was identified with probability g, until a total of $I(m)$ individuals had been identified. Very occasionally, with all units selected, $I(m)$ identifications were not achieved (usually by one to two whales). In these cases, the shortfall in identifications was not remedied.

The simulated identification histories of the individual whales were then used as input for the mark–recapture method, and new maximum-likelihood population parameter estimates (Z, λ, and μ, and hence N) were calculated. This simulation process was carried out 100 times, and approximate 95% (nonsimultaneous) confidence intervals for each original parameter estimate were obtained by discarding the five most extreme simulated estimates. The medians of these 100 simulated estimates were used as checks for possible biases in the parameter estimation.

Simulation 2

In order to test the hypothesis that $\mu = 0$ (no reimmigration), simulated populations with this property were constructed in a similar manner to that described for Simulation 1, except that an original pool of N/U units within the study area was formed 24 mo before the first month of field research (so that the number of units present in month 1 would show realistic variation). During each subsequent month, until the last month of field research, units within the study area left it with probability λ, never to return, and $n(m)$ new units entered the study area. $n(m)$ was distributed according to the Poisson distribution with mean $\lambda \cdot N$.

Simulation 3

In order to compare characteristics of identification histories between the original data set and simulated sets, it was important that during each month the same number of whales were sighted in both data sets. Therefore, we only considered the first 50 whales identified in any month if more than 50 were identified. This cutoff was chosen so that, with the simulated data sets, this number of whales could be identified without exhausting all units in the study area. The simulated few runs for which this was not achieved were discarded. No parameter estimates were made from these runs, but the distributions of the total number of months, and number of consecutive months, in which individuals were identified for the actual and simulated data sets are compared in Table 4.

Simulation 4

These simulations were designed to investigate the effects of variation in the probability of identification. The procedure was as in Simulation 1 except that, instead of being fixed at g = 0.585, g was chosen from the uniform distribution between 0.285 and 0.885. In the first set of runs, a different g was chosen for each individual, and in the second set of runs, a different g was selected for each unit and used for all whales in the unit.

Simulation 5

This simulation examined the effects of permanent, unrecognizable changes in the identification marks on individuals, as well as mortality and recruitment. The procedure was as in Simulation 1 except that the identification history of each individual, designated by the months in which it was identified (e.g. "1, 3, 24" for identified in months 1, 3, and 24), was examined. During each interval of d months between identifications ($d = 2, 21$ in the example) the whale's identification number was changed with probability $(1 - (1 - \beta)^d)$, where β is the mean rate of change in identification marks per month. For instance, if there was a change in the history "1, 3, 24" between months "3" and "24," then this would result in two new identification histories "1, 3" and "24."

Production of Domoic Acid, a Neurotoxic Amino Acid, by an Axenic Culture of the Marine Diatom *Nitzschia pungens* f. *multiseries* Hasle[1]

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Douglas, D. J., and S. S. Bates. 1992. Production of domoic acid, a neurotoxic amino acid, by an axenic culture of the marine diatom *Nitzschia pungens* f. *multiseries* Hasle. Can. J. Fish. Aquat. Sci. 49: 85–90.

A microbially contaminated culture of *Nitzschia pungens* f. *multiseries* (clone TKA-2) was treated with gentamicin followed by a combination of penicillin and streptomycin. An axenic culture was successfully isolated as evidenced by sterility tests using four different nutrient enrichment media and examination by epifluorescence microscopy. The isolate had a specific division rate ($0.65 \cdot d^{-1}$) comparable with that reported for nonaxenic cultures. The axenic culture produced domoic acid (DA) during the lag and stationary phases, when cell division rates were low or zero, but not during exponential growth. Production of DA resumed within 1 d after cell division ceased and reached 0.5 pg DA·cell^{-1}·d^{-1} 2–3 d after the onset of the stationary phase. A maximum concentration of 2.0 pg DA·cell^{-1} was reached after 6 d in stationary phase. Our results provide the first evidence that *N. pungens* f. *multiseries* produces DA in the absence of other microorganisms.

Une culture de *Nitzchia pungens* f. *multiseries* (clone TKA-2) présentant une contamination microbienne a été traitée par la gentamicine suivie d'un mélange de pénicilline et de streptomycine. Une culture axénique a pu ensuite être isolée comme l'ont démontré des tests de stérilité faisant appel à quatre milieux d'enrichissement et l'examen par microscopie d'épifluorescence. L'isolat présentait un taux de division spécifique ($0{,}65 \cdot d^{-1}$) semblable à celui signalé pour ces cultures non axéniques. La culture axénique produisait de l'acide domoïque (AD) pendant la phase de latence et la phase stationnaire, phases pendant lesquelles le taux de division est faible ou nul, mais non pendant la phase de croissance exponentielle. La production d'AD a repris un jour après la fin de la division cellulaire et atteint 0,5 pg AD·cell^{-1}·d^{-1} de 2 à 3 jours après le début de la phase stationnaire. Une concentration maximale de 2,0 pg AD·cell^{-1} a été obtenue après 6 jours de phase stationnaire. Nos résultats montrent, pour la première fois, que *N. pungens* f. *multiseries* produit de l'AD en l'absence d'autres microorganismes.

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A food poisoning event in 1987 associated with cultivated mussels from Cardigan River, Prince Edward Island, Canada, resulted in the discovery that the neurotoxin domoic acid (DA), was produced by a bloom of the pennate marine diatom *Nitzschia pungens* forma *multiseries* (Bird et al. 1988; Subba Rao et al. 1988; Bates et al. 1989; Wright et al. 1989). Because one of the characteristic neurological effects of DA ingestion by humans is loss of recent memory, the syndrome caused by this toxin has been termed "amnesic shellfish poisoning" or ASP (Todd 1990). Despite the relative abundance and worldwide distribution of *N. pungens* (Hasle 1965; Forbes and Denman 1991), there have been no reports of this alga being involved in a documented shellfish poisoning previous to the 1987 event. Of the two forms of *N. pungens*, namely f. *multiseries* and f. *pungens*, only f. *multiseries* has been confirmed to produce DA (Smith et al. 1990). Our use of "*N. pungens*" in this article refers to f. *multiseries* only.

All investigations of DA production published to date have utilized nonaxenic *N. pungens* cultures. Bacteria isolated from other marine phytoplankton species have been reported to produce "algal" toxins independently of the host algae (Kodama et al. 1988). A potential role of bacteria in the production of DA is intriguing and could explain the observations that several taxonomically diverse algal groups including red macroalgae (Impellizzeri et al. 1975) and other species of *Nitzschia* (Martin et al. 1990; Smith et al. 1990) produce DA. Although there is circumstantial evidence that heterotrophic bacteria do not control the production of this toxin (Bates et al. 1991), axenic cultures are required to establish whether there is a direct microbial role in the production of DA by cultures of *N. pungens*. The goals of this report are (1) to demonstrate that *N. pungens* can be isolated into axenic culture and (2) to show that an axenic culture of *N. pungens* produces DA.

Materials and Methods

Isolation of the Axenic Strain

The axenic strain of *N. pungens* established during this investigation was produced from a nonaxenic culture (clone TKA-2) isolated in 1989 from Galveston Channel, TX (Fryxell et al. 1990). Cultures were grown in f/2 medium (Guillard 1975) at 15°C and at a light intensity of 30–50 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a 12:12 h light–dark cycle unless otherwise indicated.

Our isolation of an axenic strain of *N. pungens* involved three sequential steps: (1) treatment with gentamicin in liquid

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medium, (2) growth of the diatom cells on agar, and (3) treatment with a combination of penicillin and streptomycin in liquid medium. A 300-mL sample of nonaxenic *N. pungens* culture in late exponential phase was concentrated onto a sterile 3.0-μm pore-size polycarbonate filter (Nuclepore Corp., Pleasanton, CA) to remove most of the free-living bacteria. The cells on the filter were resuspended in 50 mL of fresh f/2 medium. Aliquots (3 mL) of the cell suspension were treated for 48 h with the antibiotic gentamicin (Sigma Chemical Co., St. Louis, MO) at concentrations of 5, 10, 25, 50, and 100 $\mu g \cdot mL^{-1}$ in 50 mL of f/2 medium. Following this treatment, 0.5-mL samples of the culture were spread onto "super clean" agar (Waterbury et al. 1986) prepared with f/2 medium. These steps were performed under the temperature and light conditions described above.

After 21 d of incubation, there was algal growth on the f/2 agar plates which had been inoculated with cells treated at concentrations of gentamicin ≤ 50 $\mu g \cdot mL^{-1}$. Small amounts of diatom cell biomass from these plates were transferred with a sterile loop to liquid f/2 medium in flasks. These cultures were incubated for 12 d and then 10 mL of each culture was transferred to 75 mL of fresh f/2 medium. Subsamples (0.1 mL) were spread onto organic nutrient-enriched marine agar (2216 Marine Agar, Difco Laboratories, Detroit, MI) to test for the presence of heterotrophic microbial contaminants. This sterility test revealed that all the cultures were still contaminated with bacteria. However, the tests of the culture originating from the cells treated with 50 μg gentamicin$\cdot mL^{-1}$ indicated that the majority of the original contaminating microorganisms present had been killed.

A 1.0-mL sample of the viable algal culture which had been treated with 50 μg gentamicin$\cdot mL^{-1}$ was transferred to 50 mL of f/2 medium containing an antibiotic concentration series consisting of 0:0, 0.2:0.1, 0.4:0.2, 0.8:0.4, 1.6:0.8, and 3.2:1.6 mg$\cdot mL^{-1}$ penicillin-G:streptomycin (Sigma Chemical Co., St. Louis, MO). After 48 h of treatment with penicillin and streptomycin, samples containing a few (one to five) diatom cells from each antibiotic concentration were drawn from the medium into sterile glass capillaries and inoculated into 10 mL of f/2 medium in replicate (two to five) glass test tubes. The tubes were incubated examined for growth approximately every 2 d for a total of 60 d.

After 38 d, 2 of the 16 tubes showed diatom growth. One of these was a "control" sample which had not been treated with the antibiotic mixture. The cells in the second tube had been treated with 1.6 mg penicillin-G$\cdot mL^{-1}$ and 0.8 mg streptomycin$\cdot mL^{-1}$. Sterility tests (see below) of subsamples from two tubes revealed that the "control" culture included bacteria but that the treated culture had been successfully isolated from the contaminants that were originally present. Scanning electron microscopy of acid-cleaned frustules from the treated culture confirmed that the cells were of the toxin-producing diatom species *N. pungens* f. *multiseries*.

Sterility Tests

Four different organic nutrient-enriched media tests were used to determine whether microbial contaminants were present in the culture after antibiotic treatment. These were also used to test the experimental culture used to monitor DA production. Two types of organic nutrient-enriched agar were used to cultivate potential bacteria or fungi: 2216 Marine Agar and glucose – yeast extract agar, the latter being formulated specifically for cultivation of marine fungi (Kohlmeyer and Kohlmeyer 1979). Two nutrient-enriched liquid media were used: f/2 medium plus peptone (Guillard 1975) and STP (Provasoli et al. 1957). In each case, duplicate samples were spread onto agar plates and were added to liquid media in sterile test tubes. The agar and liquid test media were incubated in the dark for 30 d at both 15 and 25°C and examined at approximately 5-d intervals.

Samples of the culture after antibiotic treatment and at the end of the experiment monitoring DA production were also examined for the presence of microorganisms using acridine orange stain and epifluorescence microscopy (Hobbie et al. 1977). These preparations were made by filtering 1- to 5-mL samples onto 0.2-μm pore-size polycarbonate membrane filters prestained with Irgalan black (Nuclepore Corp., Pleasanton, CA). The filters were then stained with acridine orange (0.05%, Sigma Chemical Co., St. Louis, MO), mounted in immersion oil on microscope slides, and examined at 1250× magnification under epifluorescent illumination with a Zeiss Universal microscope.

Culture Growth System

The *N. pungens* culture used to monitor DA production was grown in a 12-L fermentor system (Microferm, New Brunswick Scientific, New Brunswick, NJ) which was modified to include supply and measurement of light for photosynthesis. The system consisted of a glass vessel with a stainless steel headplate assembly and contained probes for automatic measurement and recording of temperature, pH, stirring rate, aeration rate, and photosynthetically active radiation (PAR). Temperature was controlled by thermostatically regulated flow of water through stainless steel baffles extending into the culture medium. The culture was stirred at 75 rpm and was bubbled with air ($0.45\ L \cdot min^{-1}$) which was steam sterilized and sterile filtered (0.22-μm filter; Pall Trinity Micro Co., Cortland, NY). Light was supplied on a 12:12 h light–dark cycle by circular Cool-White fluorescent tubes which surrounded the culture vessel. Neutral density screens were used to attenuate the light intensity to approximately 100 $\mu E \cdot m^{-2} \cdot s^{-1}$, measured by a light meter equipped with a 4π sensor (model QSL-100, Biospherical Instruments, San Diego, CA) located inside the vessel.

Ten litres of f/2 medium buffered with 4.1 mM Tris buffer (tris(hydroxymethyl)aminomethane) was added to the culture vessel. The system was then steam sterilized in a large autoclave. The next day, the temperature of the growth medium was brought to 15°C and the pH of the medium was adjusted to 7.7 by a supply of sterile CO_2. The system was then inoculated with 800 mL of an 11-day-old axenic culture of *N. pungens* in early stationary growth phase using sterile technique in a laminar flow hood equipped with a high-efficiency particle retaining air (HEPA) filter. Samples were withdrawn daily by a peristaltic pump through steam-sterilized silicone tubing

Cell Numbers and Optical Density Measurements

Cell numbers of *N. pungens* were determined from two to four replicate microscope cell counts (30–700 cells per count) of 5-μL samples. The optical density (OD) of culture aliquots was measured at 750 nm (model PC 800 colorimeter, Brinkmann Instruments, Westbury, NY). The specific growth rate was calculated from microscope cell counts obtained during the exponential growth base.

Changes in cell number were also estimated by in situ measurements of the change in light intensity reaching the PAR light meter during growth of the culture. The PAR-based OD was determined from the decrease in percent transmittance of PAR by the same calculation used for conventional single wavelength light OD measurements (e.g. Sorokin 1973).

DA Analysis

The concentration of DA in 20-mL subsamples of the whole culture (cells plus culture medium) was measured as follows. Samples of the culture were withdrawn from the sampling port and frozen immediately at −20°C. At the time of analysis, the samples were thawed, sonicated, and filtered to remove particulate material. The DA in the resulting filtrate was determined using high-performance liquid chromatography (HPLC) of the fluorenylmethoxycarbonyl derivative (Pocklington et al. 1990). Analyses were also conducted on several samples using HPLC with ultraviolet diode array detection (Quilliam et al. 1989) to provide independent spectroscopic confirmation of the identity of DA. The DA concentration per cell was calculated by dividing the concentration of DA in the whole culture by the microscope cell counts, and rates of DA production were calculated by dividing the daily incremental increases in DA concentration by the cell counts.

Results

Tests for Microbial Contaminants

The original nonaxenic culture included both unidentified bacteria and microflagellates. Following the final antibiotic treatment, all agar and liquid enrichment tests for bacterial and fungal contaminants in the stock culture were negative. The results of sterility tests conducted on the experimental culture at the time of inoculation and at the end of the experiment were also all negative. Tests of nonaxenic cultures of *N. pungens* provided unequivocal positive evidence of the presence of microbial contaminants, usually within 5 d after inoculation.

Epifluorescence microscopic examination of acridine orange stained samples of the stock culture after the antibiotic treatments showed no evidence of identifiable bacteria, fungi, or microflagellate cells. Similarly, microscopic examination of the experimental culture during and at the end of the experiment indicated that the cultures were free of microbial contaminants.

Cell Growth

Microscope cell counts showed that the axenic culture of *N. pungens* had an initial lag period of approximately 3 d followed by exponential growth for approximately 6 d (Fig. 1A). The specific division rate was $0.65 \cdot d^{-1}$. The total number of diatom cells was nearly constant after 10 d. Microscopic examination of samples from the aging experimental culture, however, suggested a gradual decline in the numbers of "healthy" cells, as indicated by an increasing proportion of cells with little or no pigmentation after several days of stationary phase. Daily OD measurements made at 750 nm showed the same growth pattern as the microscope cell counts (Fig. 1B) as evident from the significant correlation of OD data with cell counts (Pearson correlation coefficient, $r = 0.998$, $n = 15$, $p < 0.001$).

Diatom cell growth was also estimated from the decrease in transmittance of PAR measured within the culture vessel (Fig. 1C). Overall, the pattern of cell growth obtained from PAR-based data was in close agreement with the measurements of cell numbers and OD at 750 nm (Fig. 1B and 1C). However, during the stationary phase there was a significant difference between estimates of cell abundance based on OD measurements made at 750 nm compared with estimates from PAR. The OD measurements at 750 nm indicated that cell numbers remained nearly constant, while the PAR-based OD estimates indicated a substantial decrease in cell biomass after several days in stationary phase, consistent with decreasing numbers of healthy cells.

Production of DA

DA was produced by the axenic strain of *N. pungens* under controlled culture conditions (Fig. 1D and 1E). DA production was closely coupled to the diatom culture growth phase (Fig. 1D). This relationship was most evident when the increase in DA in the culture was related to diatom cell number; production rate during the lag and stationary phases reached similar values of approximately 0.5 $pg \cdot cell^{-1} \cdot d^{-1}$ and was essentially zero during exponential growth (Fig. 1E). Cells used for the inoculum were in early stationary phase and were producing DA at the time of inoculation (time zero, Fig. 1E). This production continued during the lag phase when there was a negligible increase in cell number. Both the rate of production of DA per cell and the concentration of DA per cell rapidly decreased during exponential growth (Fig. 1E), the latter due to an increase in cell number. The production of DA resumed within 1 d after cell division ceased, and the rate of DA production reached 0.5 $pg \cdot cell^{-1} \cdot d^{-1}$ within 2–3 d after the cells stopped dividing. After this, the rate of DA production began to decline (Fig. 1E). After 6 d in stationary phase, the DA concentration in the culture reached 290 $ng \cdot mL^{-1}$ (Fig. 1D), corresponding to 2.0 pg $DA \cdot cell^{-1}$ (Fig. 1E).

Discussion

The first goal of our study was to isolate an axenic strain of the neurotoxin-producing diatom *N. pungens*. Isolation of axenic cultures of algal species can be problematic and there is no single method applicable for all situations (e.g. review by McCracken 1989). Some algal species have proven to be difficult or even impossible to bring into culture in the absence of associated microorganisms (Droop and Elson 1966). The problems facing the investigator usually include the abundance, coupled with short generation times, of the contaminating microorganisms relative to the algae.

Antibiotic treatment has become one of the most widely used methods, largely due to the specificity of action on prokaryotic versus eukaryotic cell types. In one of the first published attempts to produce an axenic strain of a diatom (*N. closterium* f. *minutissima*), Spencer (1952) used penicillin and streptomycin, both singly and in combination, with an exposure time of 16 d. These attempts proved unsuccessful, as concentrations which killed bacteria also inhibited diatom growth. Later, Droop (1967) showed that a shorter exposure time with high concentrations of antibiotics was more likely to lead to successful isolation.

In our study, the sequence of treatment with gentamicin, followed by penicillin and streptomycin, was chosen after preliminary experiments indicated that neither gentamicin nor penicillin/streptomycin alone killed all of the bacteria present in the original culture. It should be noted that another culture of

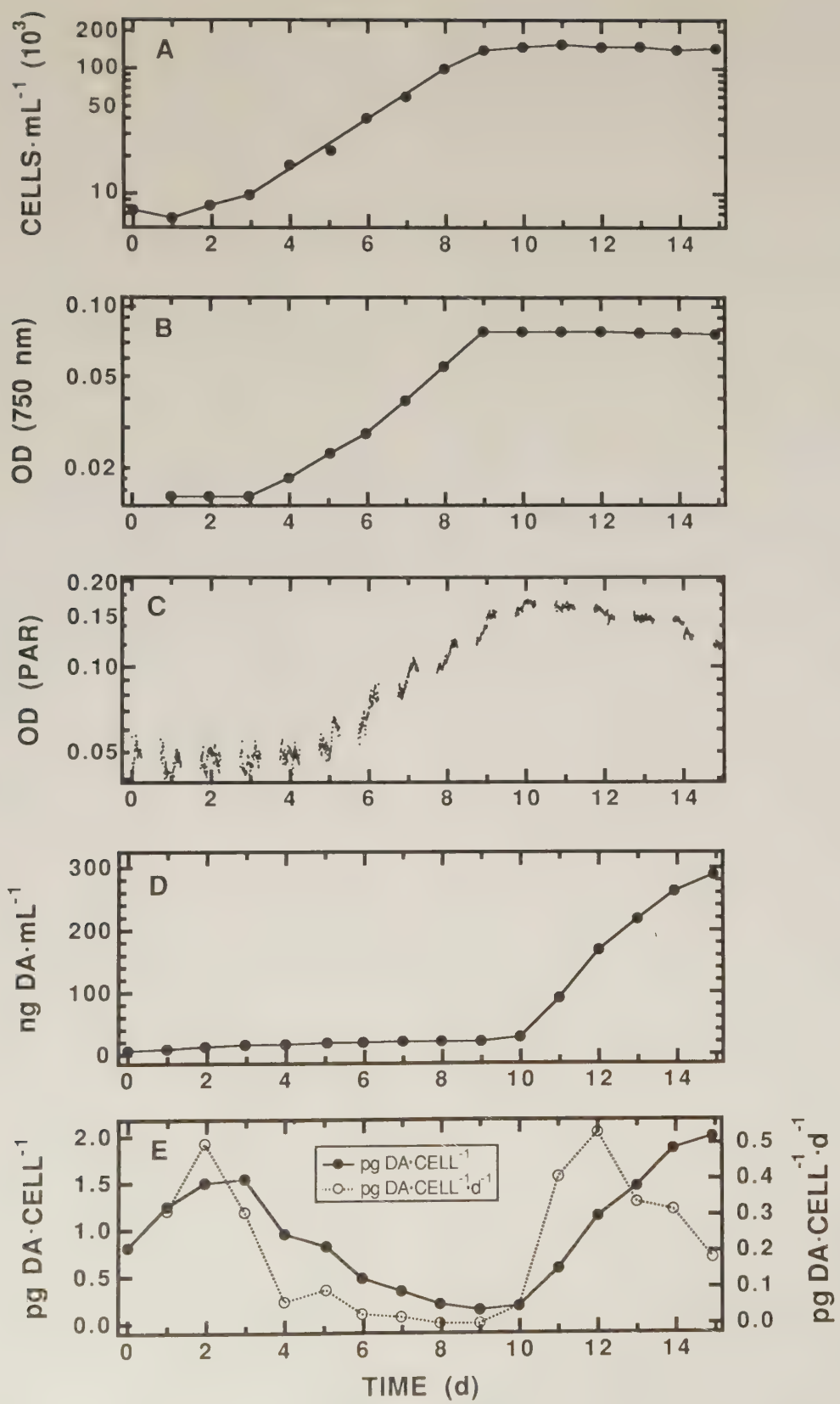

FIG. 1. Growth and production of DA by an axenic culture of *N. pungens* f. *multiseries*. (A) Cell numbers; (B) OD at 750 nm; (C) OD based on PAR; (D) DA concentration in the whole culture; (E) DA concentration in the whole culture related to cell numbers (DA)·cell^{-1}; solid circles) and rate of DA production related to cell numbers (DA·cell^{-1}·d^{-1}; open circles).

N. pungens, originating from Prince Edward Island (clone NPARL; Bates et al. 1989), has also been successfully rendered axenic using a penicillin/streptomycin combination (R. Selvin, Bigelow Laboratory for Ocean Science, West Boothbay Harbor, ME, pers. comm.) and probably initially contained bacteria with different antibiotic sensitivities than our culture which originated from Galveston Channel, TX.

For this study, it was critical to ensure that our culture was axenic. There is, however, no one universally accepted medium or protocol for detecting microbial contaminants in algal cultures (e.g. Provasoli et al. 1957; Hoshaw and Rosowski 1973; Guillard 1975). We employed four different types of nutrient-enriched media formulated specifically for isolation and growth of bacteria and fungi. To increase the likelihood of detecting potential contaminants with narrow temperature requirements, we conducted duplicate test series at 15 and 25°C. To examine the possibility that the culture contained contaminants that did not grow on the nutrient-enriched media, the cultures were further examined using acridine orange staining and epifluorescence microscopy. The use of epifluorescence is particularly well suited for detecting small bacteria which are typical in aquatic samples (Hobbie et al. 1977). The results of epifluorescence microscopy, combined with the results of the nutrient enrichment tests, support the conclusion that the algal cells had been successfully isolated from the microbial contaminants originally present in the culture.

Our second goal was to determine if *N. pungens* produced DA in the absence of other microorganisms in the culture. Our results establish that an axenic culture of this diatom does, in fact, produce DA. Furthermore, our DA production data fall within the range of values (0.3–8 pg DA·cell^{-1}) reported from nonaxenic cultures (Subba Rao et al. 1988, 1990; Bates et al. 1989, 1991). The timing of DA production relative to the growth phase of the culture, from essentially no production during exponential phase to an initiation of DA production immediately after the beginning of stationary phase, is also the same as reported for nonaxenic cultures of *N. pungens* (Subba Rao et al. 1988, 1990; Bates et al. 1989, 1991). That there was also DA production during the lag phase when all nutrients were in excess suggests that toxin production may be a general response at times of zero cell division. Our results indicate that there was a minimal carryover of any potential antibiotic effects on the metabolic control of DA production. The results also suggest that the production of DA observed in previous studies of nonaxenic cultures of *N. pungens* can be attributed to algal biosynthesis and that bacterial involvement was not essential.

To our knowledge, this is the first study to use electronically sampled in situ PAR measurements to characterize the growth of phytoplankton in a sterilizable culture system. The PAR-based method has several important advantages which include unattended sampling, a finer temporal resolution of growth (Fig. 1C versus Fig. 1B), no loss of culture volume associated with sampling, and no chance of introducing contaminants into the culture.

Overall, the results of PAR-based OD were in close agreement with cell numbers and single wavelength OD measurements at 750 nm using samples removed from the culture system (Fig. 1A, 1B, and 1C). One significant difference between the PAR- and 750-mm-based OD measurements occurred several days after the onset of stationary phase. At this time, the PAR-based OD measurements indicated a loss of cell abundance (Fig. 1C) although the 750-nm OD values remained relatively constant (Fig. 1B). This difference probably reflected the sensitivity of the PAR sensor to decreases in light absorption from pigments in the culture as it became senescent. OD measured at 750 nm appeared to be relatively independent of changes in pigmentation and provided a good indication of total algal cell numbers (Sorokin 1973). The PAR-based values, however, appear to be more indicative of the number of healthy, viable cells. This conclusion was strengthened by the increase in the proportion of senescent cells in the experimental culture through the later stationary phase as observed by microscopic examination. This result may be relevant to interpreting the data on DA production related to microscope cell counts, which indicate a marked decline in the DA production per cell several days after the onset of stationary phase (Fig. 1E). This decline may have been caused, at least in part, by a decreasing number of DA-producing cells in the culture during late stationary phase.

Our finding that DA is produced by an axenic culture of *N. pungens* is especially significant, as there is currently considerable interest in the possible role of bacteria in algal toxin production (e.g. Kodama et al. 1988; Tamplin 1990). Our results are consistent with recent reports that dinoflagellates continue to produce toxin after isolation into axenic culture (Boczar et al. 1988; Bomber et al. 1989). It is notable that the series of reports by Kodama and co-workers on toxin production by marine microorganisms associated with algae were all based on bacteria isolated from within the cells of the marine dinoflagellate *Protogonyaulax tamarensis* (Kodama et al. 1988; Ogata et al. 1990). Investigation of *N. pungens* f. *multiseries* using transmission electron microscopy has shown no evidence of internal microorganisms in this diatom species (MacPhee et al. 1992).

Finally, it has been well documented that the interactions between bacteria and algae include mechanisms which support algal growth (e.g. review by Cole 1982). Our results do not preclude the possibility that microorganisms associated with *N. pungens* may enhance diatom growth and production of DA, both in natural environments and in nonaxenic cultures. Our results establish, however, that microbial involvement is not essential for growth or for DA production by *N. pungens* f. *multiseries*.

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Benthic Boundary Layer Macrofauna of Browns Bank, Northwest Atlantic, as Potential Prey of Juvenile Benthic Fish

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Wildish, D. J., A. J. Wilson, and B. Frost. 1992. Benthic boundary layer macrofauna of Browns Banks, northwest Atlantic, as potential prey of juvenile benthic fish. Can. J. Fish. Aquat. Sci. 49: 91–98.

A first quantitative description is provided of the drifting or swimming macrofauna present within the benthic boundary layer (BBL) over the hard sediments of Browns Bank in the northwest Atlantic. Major categories of identified animals include drift epi- or infauna, zooplankters, fish larvae and eggs, and suprabenthic animals. Suprabenthic amphipods were good indicators of the type of sediment/flow environment that they were associated with. Although zooplanktonic copepods were by far the most abundant and suprabenthos the most diverse group of the BBL macrofauna, they appear to be ignored as a source of food by juvenile haddock (*Melanogrammus aeglefinus*), which are known to feed on prey classified herein either as in situ, or drifted, epi- or infauna. Unfortunately, the BBL sled used in our study could not sample animals at <33 cm from the sediment–water interface. Our results imply that juvenile haddock feed either directly on animals living in, or at the sediment interface, or on drifting animals present within the BBL at heights <33 cm above the bottom

Les auteurs présentent l'une des premières descriptions quantitatives de la macrofaune dérivante ou nageante se trouvant dans la couche limite benthique sus-jacente aux sédiments durs du banc Browns, dans le nord-ouest de l'Atlantique. Les principales catégories d'animaux décelés étaient des organismes d'épi-ou d'endofaune, du zooplancton, des larves et des oeufs de poissons et animaux suprabenthiques. Les amphipodes suprabenthiques étaient de bons indicateurs du type d'environnement des sédiments ou de l'écoulement avec lequel ils étaient associés. Les copépodes zooplanctoniques étaient de beaucoup les plus abondants et ceux du suprabenthos les plus diversifiés de la macrofaune de la couche, mais ces organismes semblent être délaissés par les aiglefins (*Melanogrammus aeglefinus*) juvéniles que l'on sait se nourrir de proies de l'épi- ou de l'endofaune in situ ou en dérive. Malheueursement, le traîneau d'échantillonnage de la couche limite utilisé ne permettait pas d'échantillonner les animaux se trouvant à moins de 33 cm de l'interface sédiments–eau. Nos résultats portent à croire que les aiglefins juvéniles se nourrissent directement à partir des animaux vivant dans la couche ou présents à l'interface des sédiments ou à partir des animaux en dérive présents dans la couche à moins de 33 cm du fond.

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This research was undertaken as part of an interdisciplinary ecological study of the haddock (*Melanogrammus aeglefinus*), particularly the demersal juvenile stage which is a benthic visual predator (Brunel 1968; Kane 1984; Mahon and Neilson 1987). We were interested in identifying all macrofauna present within the benthic boundary layer (BBL) (see Vogel 1981) on, and near, Browns Bank, northwest Atlantic, which is reported to be a feeding ground for juvenile haddock (Mahon and Neilson 1987; J. D. Neilson, pers. comm.) Our aim was to provide quantitative estimates of density and biomass for BBL macrofauna of potential value as prey items for the early life history stages of fishes such as the haddock.

Previous studies of macrofauna swimming, or passively carried, within the BBL of continental shelves have usually been conducted over soft sediments (Bossanyi 1957; Brunel 1968, 1979; Fosså 1985; Kaartvedt 1985; Saint-Marie and Brunel 1985). Our sampling was concentrated over the hard, sometimes rocky Sambro sand or Sable Island sand or gravel sediments of the Browns Bank area (Fig. 1), except for two stations within the Roseway Basin, a deeper area with silt–clay depositional sediment (Fader et al. 1977). In such situations the BBL macrofauna are not representatively sampled by conventional grabs (Wildish et al. 1989) or in conventionally towed plankton nets (Sameoto 1982; Tremblay and Roff 1983), so our study is the first to quantitatively sample the hard Scotian Shelf BBL off eastern Canada in the northwest Atlantic.

Materials and Methods

Benthic Boundary Layer (BBL) Sampler

The BBL sled (Fig. 2) used throughout this work was based on the Macer-GIROQ epibenthic sled sampler of Brunel et al. (1978). The original sampler was designed for work on soft sediments. Our modifications improved the stability and strength of the sled for work on the harder and rocky bottoms of the Scotian Shelf banks. Modifications incorporated in the BBL sled included a roll bar framework which protected the nets and boxes, installation of lateral fins at 15° from the horizontal, to act as a depressor during fishing and with fins set at a positive dihedral angle of 20° to keep the front of the sled up during deployment, and provision for access to the flowmeter through the box wall. The construction was of heavy steel for the runners and toboggan bottom plate and marine-grade aluminum for the upper sled to produce a low centre of gravity, thus enhancing stability during towing. The lower sampling box (Fig. 2, V) could be adjusted for height above the sediment–water interface (range 15–45 cm above it) with

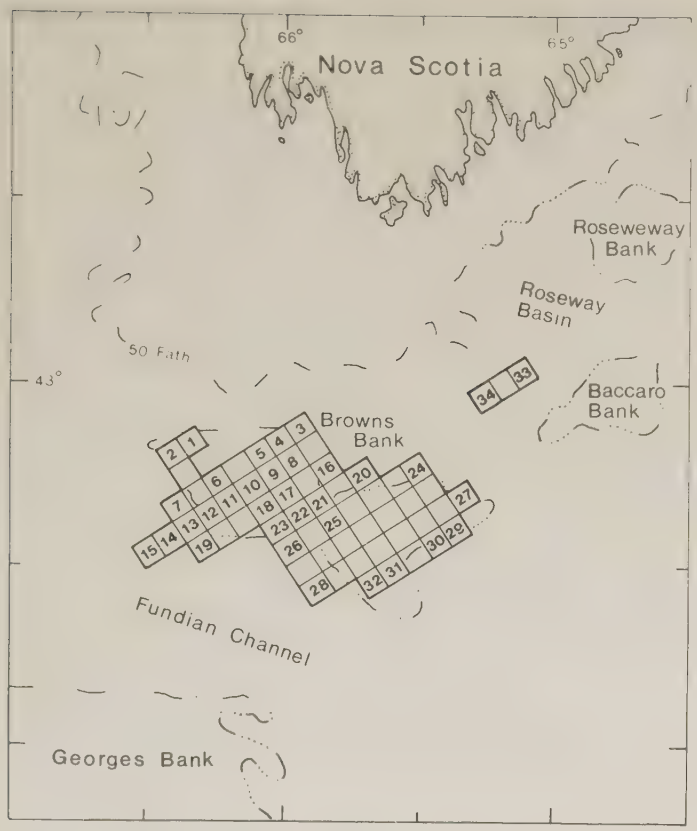

FIG. 1. Map showing the 34 sampling stations used during this study. Station coordinates at the commencement of towing are available on request.

the setting used througout this work being 33 cm above it to minimize flow interference by the upturned part of the toboggan plate.

The BBL sled had an overall length of 240 cm, width of 125 cm, and height of 160 cm. With the lower box adjusted to its lowest position, the centre of gravity was 46 cm from the bottom toboggan plate. In air the sled weighed approximately 480 kg, exclusive of flowmeter and nets. The BBL sled sampler was fabricated by Bourque Manufacturing Ltd., P.O. Box 2659, Saint John, N.B. E2L 4Z1, Canada, who also have working drawings of the sampler and can manufacture additional versions on request.

The samplers were 2-m-long plankton nets constructed of 0.5-mm-mesh Nitex attached to the boxes by hook and eye and pulley and wire attached to the frame (Fig. 2 W and g). The boxes were adjusted to sample at 33–73 and 109–149 cm above the sediment–water interface throughout this study. The degree of upturn of the toboggan plate is dictated by the rock sizes encountered on Browns Bank and this in turn limited sampling to >33 cm above the sediment. The upper limit of 149 cm sampled was chosen so that the BBL was adequately sampled. During sampling, flow into each box was recorded by a TS flowmeter (Tsurumi-Seiki Co., Ltd., Japan) attached to the wall. Sampling boxes were open and plankton nets sampled, as recorded by the flowmeter, only when the central lever (Fig. 2, d) was depressed by its contact with the bottom.

Field Sampling

Fishing with the BBL sled was either from the research trawler *E. E. Prince* or from the stern of the research vessel *J. L. Hart*. The warp to depth ratios used to deploy the sled were generally of the order 3 to 1 for depths in excess of 90 m and 2 to 1 for depths of <25 m. Each sample consisted of a 20-min tow with the vessel proceeding at approximately 2 knots.

The number of revolutions on each flowmeter was noted (Fig. 2 h). The nets were washed down with seawater and the codend contents transferred to plastic buckets. Formalin was added to make a 5% solution in seawater, the bucket labelled, and an air-tight lid attached.

Swimming or passively carried macrofauna at the two levels mentioned were sampled at 34 stations on, or near, Browns Bank during 1984–85 (Fig. 1). We selected stations to correspond to the 7 × 7 km grid of the Greenberg (1983) model because data on tidal currents, sedimentary conditions, and benthic macrofaunal production were already available for some of the grid boxes (see Wildish et al. 1989). However, the sampling station numbers used herein do not correspond to those used in the earlier report. Geographic sampling involved two cruises on 31 May to 6 June and 18–24 July 1985. The two deep Roseway Basin stations (Fig. 1, Nos. 33 and 34) were sampled on 25 November 1984. Five stations (Fig. 1, Nos. 6, 7, 8, 20, and 28) were seasonally sampled during 1984–85. Sampling periods in 1984 were 15–20 May, 22–26 June, and 23–27 November. One sampling period in 1985, additional to the two already mentioned, was 4–10 April. All of the sampling was during 08:00–21:00.

TABLE 1. Density and biomass of upper and lower net samples from Browns Bank during summer 1985.

Station No.	Upper net		Lower net	
	no/m^3	mg/m^3	no./m^3	mg/m^3
1	11.60	4.45	17.70	3.93
2	42.22	23.14	40.47	20.07
3	20.35	14.86	21.07	108.63
4	1.21	1.07	1.56	1.29
5	25.55	14.46	16.00	9.46
6	78.64	130.00	69.08	69.76
7	2.47	1.01	1.75	2.75
8	16.18	5.99	9.93	4.88
9	5.07	19.47	9.05	26.67
10	47.23	22.79	19.07	9.04
11	20.59	6.88	44.60	17.80
12	8.11	4.78	11.96	6.62
13	14.59	7.50	15.30	13.40
14	11.96	5.07	11.68	13.73
15	1.29	0.93	11.50	11.70
16	36.73	35.82	26.60	29.55
17	25.61	7.90	24.67	12.24
18	14.17	6.36	8.88	11.16
19	366.02	248.43	264.49	241.25
20	61.46	48.33	29.73	20.63
21	6.86	4.77	5.09	1.93
22	24.97	8.01	20.84	6.09
23	13.62	9.11	3.47	1.49
24	17.10	11.34	3.44	2.69
25	15.11	12.67	19.32	14.06
26	11.40	7.46	0.91	0.75
27	1.53	1.01	2.20	2.30
28	2.77	1.79	7.41	6.05
29	67.73	54.80	25.92	20.68
30	22.02	11.67	0.09	0.11
31	4.68	0.90	533.20	300.04
32	108.54	66.87	28.41	13.64
33	9.26	9.55	0.10	0.40
34	3.39	2.73	4.80	16.01

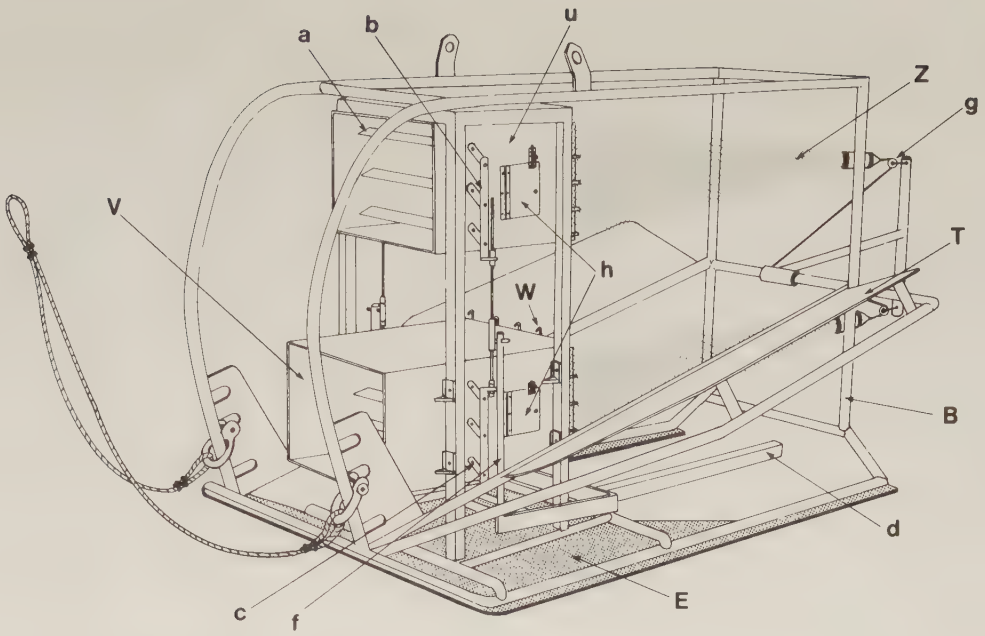

FIG. 2. Benthic boundary layer (BBL) sled. The letters shown refer also to the original Macer-GIROQ sled shown in Holme and MacIntyre (1984, p. 148). B, roll bar cage of tubular 35-mm iron pipe; E, toboggan bottom plate of 5-mm-thick A36 steel; T, marine-grade aluminum fin of 120 × 40 cm × 5 mm, adjustable between 0 and 15° and fixed deflection angle of 20°; u, aluminum box of upper fixed net; V, aluminum box of lower, adjustable net (the lower box edge can be 15–45 cm above the sediment–water interface); W, hooks for attachment of the eyes of the plankton net; Z, plankton net; a, shutter closing mechanism; b, adjustable control link; c, crank lever; d, central lever operating the closing mechanism; f, closing rod; g, pulley block and line for securing the plankton net; h, door for servicing the flowmeter.

TABLE 2. Most common BBL taxa in 34 samples from Browns Bank area in June and July 1985.

Taxa	Max. body length or diam. (mm)	Frequency of occurrence in all nets (%)	Mean biomass (mg/m^3)		Mean density ($no./m^3$)		
			Upper	Lower	Upper	Lower	*K*
Calanus finmarchicus	6.5	100	17.34	20.41	25.5	31.1	0.45
Metridia sp.	4.5	90	0.61	0.39	1.1	0.8	0.58
Sagitta elegans	20	79	0.34	0.47	0.1	0.2	0.33
Fish eggs	2	69	0.05	0.08	0.1	0.3	0.25
Pagurus sp. larvae	2	65	0.08	0.09	0.1	0.1	0.50
Metopa bruzelli[a]	8	63	0.03	0.09	0.1	0.3	0.25
Calanus sp.	6.5	62	0.08	0.05	0.2	0.2	0.50
Eualaspusiolus sp. larvae	5	57	0.08	0.05	0.10	0.07	0.59
Pseudocalanus minutus	1.6	54	0.39	0.62	1.79	3.04	0.37
Pandalus montagui	100	51	0.11	0.61	0.06	0.05	0.55
Balanus sp. larvae	1.5	46	0.11	0.15	0.72	1.00	0.42
Petalosarsa declivis	5	46	0.01	0.02	0.02	0.03	0.40
Hydrozoa	—	46	0.04	0.28	0.01	0.03	0.25
Metopa sp.[a]	8	40	0.01	0.03	0.05	0.10	0.33
Calanus hyperboreus	5	38	0.22	0.19	0.17	0.10	0.63
Oikopleura sp.	6	37	0.04	0.08	0.07	0.04	0.64
Invertebrate eggs	0.4	35	0.03	0.01	1.30	1.28	0.50
Hydrozoa medusae	—	34	1.00	0.50	4.63	2.75	0.63
Monoculodes edwardsii[a]	8	34	0.10	0.08	0.24	0.13	0.65
Caligus elongatus	20	31	0.04	0.02	0.03	0.01	0.75
Ischyrocereus sp.[a]	10	31	0.01	0.02	0.03	0.02	0.60
Metopa pusilla[a]	8	29	0.04	0.01	0.01	0.02	0.33
Erythrops erthopthalma	11	29	0.02	0.06	0.06	0.06	0.50
Gastropoda	—	29	12.62	6.07	7.40	6.23	0.54
Ophuroidea	—	29	0.01	0.08	0.01	0.02	0.33
Euphausidae	40	29	0.01	0.01	0.01	0.02	0.33
Rhacotropis oculata[a]	12	26	0	0.17	0	0.03	—
Sagitta seratodentata	16	25	0.15	0.08	0.05	0.03	0.63

[a]Gammaridean amphipods.

TABLE 3. Comparison of BBL sled net catches for species present in 5 or more nets on and near Browns Bank. The average density ($\bar{x}$/m^3) and standard error (SE) are given for each of the 34 nets possible at each level. The number of nets catching that species is indicated by n. Upper and lower net densities differ significantly ($p \leq 0.05$) as indicated by asterisks.

Group	Species	Upper net			Lower net			
		$\bar{x}$/m^3	SE	n	$\bar{x}$/m^3	SE	n	p
Cumacea	*Pseudoleptacuma minor*	0.0007	0.0006	2	0.0029	0.0014	7	0.18
	Eudorella truncatula	0.0003	0.0002	2	0.0058	0.0050	4	0.28
	Lamprops quadriplicata	0.0002	0.0002	1	0.0011	0.0024	6	0.07
	Diastylis sculpta	0.0016	0.0008	5	0.0034	0.0087	9	0.27
	Petalosarsa declivis	0.0038	0.0017	8	0.0236	0.0066	23	0.005*
Amphipoda	*Parathemisto gaudichaudi*	0.0017	0.0007	8	0.0004	0.0017	2	0.09
	Rhacotropis oculata	—	—	0	0.1290	—	16	—
	Stenopleustes inermis	0.0002	0.0002	1	0.0044	0.0022	11	0.06
	Metopa bruzelli	0.0339	0.0093	17	0.2291	0.0846	24	0.03*
	M. pusilla	0.0011	0.0014	6	0.0076	0.0152	14	0.02*
	M. propinqua	—	—	0	0.0239	—	6	—
	M. invalida	0.0003	0.0003	1	0.0142	0.0074	9	0.06
	Metopa sp.	0.0127	0.0086	9	0.0453	0.236	14	0.20
	Monoculodes edwardsii	0.0647	0.0455	9	0.0470	0.0249	12	0.73
	Unciola irrorata	0.0002	0.0002	1	0.0021	0.0011	6	0.10
	Erichthonius rubricornis	0.0003	0.0013	2	0.0026	0.0041	11	0.003*
	Ischyrocerus sp.	0.0028	0.0023	3	0.0121	0.0044	18	0.07
	Dulichia falcata	—	—	0	0.0018	—	11	—
	Dyopedos monocantha	0.0004	0.0015	2	0.0020	0.0009	6	0.09
	Paradulichia typica	—	—	0	0.0013	—	7	—
	Gitanopsis bispinosa	—	—	0	0.0013	—	5	—
	Syrrhoe crenulata	0.0015	0.0008	5	0.0036	0.0015	8	0.23
	Aeginina longicornis	0.0002	0.0009	1	0.0037	0.0120	9	0.09
Copepoda	*Calanus hyperboreus*	0.0728	0.0342	15	0.0318	0.0130	11	0.27
	C. finmarchicus	28.5475	15.4804	34	22.8391	9.7305	34	0.75
	Calanus sp.	0.1240	0.0475	20	0.1018	0.1819	18	0.70
	Pseudocalanus minutus	1.0234	0.5324	20	1.6680	0.7920	17	0.49
	Gaidius tenuispinus	0.0080	0.0045	4	0.0118	0.0071	6	0.66
	Temora longicornis	0.0225	0.0148	5	0.0024	0.0016	3	0.18
	Metridia sp.	0.8505	0.4394	26	0.5820	0.2345	25	0.59
	Pleurogamma gracilis	0.134	0.0083	5	0.0157	0.0077	7	0.84
	Caligus elongatus	0.0089	0.0045	12	0.0030	0.0012	9	0.21
	Aethentha depressa	0.0015	0.0013	3	0.0094	0.0056	7	0.17
Decapoda	*Erythrops erthopthalma*	0.0105	0.0095	6	0.0211	0.0689	13	0.49
	E. microps	0.0030	0.0018	3	0.0762	0.0446	5	0.11
	Meganyctiphanes norvegica	0.0359	0.0141	9	0.0579	0.0252	3	0.45
	Eulalus pusiolus	0.0579	0.0252	19	0.0359	0.0141	17	0.45
	Dichelopandalus leptocerus			0	0.0036	—	8	—
	Pandalus montagui	0.0289	0.0141	21	0.0266	0.0093	18	0.89
	Axius serratus	0.0033	0.0013	12	0.0012	0.0009	3	0.19
	Pagurus sp.	0.0830	0.360	20	0.0921	0.0663	23	0.90
	Hyas coarctatus	0.0076	0.0035	7	0.0033	0.0016	6	0.27

Laboratory Analysis

Bucket contents were collected on a 0.5-mm-mesh Nitex filter, washed with tap water, and placed in a large Petri dish. Macrofauna larger than approximately 2 mm were removed and placed in vials containing 30% ethanol for identification. The remaining material was serially split until fractions of approximately 200 individuals were obtained. A randomly chosen fraction was subsequently sorted and preserved in 30% ethanol for later identification. Representative taxa were either confirmed or identified by the Atlantic Reference Centre, Huntsman Marine Science Centre, St. Andrews, N.B., Canada. A collection of specimens from Browns Bank is curated at the Atlantic Reference Centre.

Ethanol-preserved animals which had been identified were counted and then soaked for 5 min in tap water, centrifuged at low speed, and the wet biomass determined to the nearest 1 mg. From the net opening dimensions of 40 × 55 cm and flow records made during sampling we calculated the total volume of seawater filtered by the 0.5-mm-mesh plankton net during each sampling as ranging from 111 to 444 m^3. Densities or net biomass were expressed as number or wet weight in milligrams per cubic metre. Where average values are calculated for individual species, we have excluded samples with zeros, although zeros have been included in comparing upper and lower net average densities by ANOVA. We also calculated the swimming activity measure, K, of Sainte-Marie and Brunel (1985) as

$$K = \frac{\text{Upper net density}}{\text{Upper and lower net density}}.$$

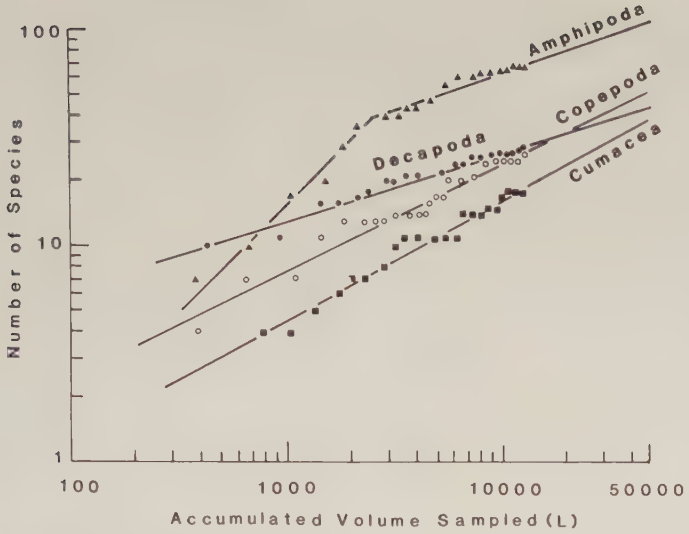

FIG. 3. Logarithmic plot of number of species against accumulated volume of seawater sampled for 32 samples taken in June–July 1985.

Low values of K indicate more individuals in the lower net, whilst $K = 0.5$ indicates equal numbers in upper and lower nets.

Results

Spatial Survey

The average biomass in the upper net (28.88 ± 8.08 mg/m^3, mean ± standard error) was not significantly different ($p = 0.65$) from that in the lower net (30.01 ± 11.13 mg/m^3) when all 34 geographical samples were pooled as replicates (Table 1). The result is due to the large variance to mean ratio characteristic of the data. Similarly, for density the mean upper net (32.94 ± 10.93 animals/m^3) was not significantly different than the mean lower net (38.54 ± 16.86 animals/m^3) contents.

A total of 126 species and 78 incompletely identified taxa[1] were found by geographical and seasonal sampling within the benthic boundary layer. The greatest biomass and abundance within geographical samples was due to the calanoid copepod *Calanus finmarchicus* (Table 2). This species was the only one present in all of the nets used in sampling. Its distribution was very patchy, ranging from 0.18 to 525.97 *C. finmarchicus*/m^3. As an aid to indicate their potential suitability as prey for benthic fish, the maximum body size of each taxon is given in Table 2. Low K values, indicating greater availability in the lower BBL, were found for some species in Table 2, e.g. gammaridean amphipods with the exception of *Ischyrocerus* sp.

We compared upper and lower net catches for individual species where five or more replicate nets were available from the pooled geographical samples (Table 3), yielding a total of 42 taxa. Comparing the mean values (with zeros included) by ANOVA ($n = 68$) showed that some species of cumaceans and amphipods had more individuals in the lower net. Of these, one species of cumacean and three species of amphipod had significantly more individuals in the lower net. Five species of amphipod (Table 3) were found only in the lower net and hence follow the same pattern of low K values. *Monoculodes edwardsii* does not follow the cumacean/amphipod pattern and statistically has equal numbers in each net. In the case of *Parathemisto gaudichaudi* which also is equally represented in each net, this may be because it is a pelagic species. By contrast, the copepods and decapods all have similar means between upper and lower nets. One exception is the decapod *Dichelopandalus leptocerus*, with all catches in the lower net. Virtually the same species of copepods and decapods were found in each net, indicating an active swimming within the BBL, whereas for the cumaceans and amphipods, other species, including many not shown in Table 3, were found only in the lower net.

The high species diversity of the amphipods (Fig. 3) and their localized geographic distribution (Table 4) make them good indicators of the type of sediment present as independently described by Fader et al. (1977) and, consequently, of the flow environment of that sampling station. The localized distribution of amphipods may be the explanation for the inflection in the species/volume plot of Fig. 3. Typical Browns Bank amphipods include *Metopa* spp. (with *M. bruzelli* dominant), *Pleustes panoplus* and *Rhacotropis oculata*, versus *Metopella* sp., *Rhacotropis distincta, Halirages inermis, Synchelidium tenuimanum*, of the poorly sorted silt–clay of Roseway Basin; and *Gitanopsis bispinosa* and *Tryphosa* sp., of the Fundian Channel (Fig. 1).

At both Browns Bank and Fundian Channel stations (Table 4), copepod densities were highest in the lower net whereas in Roseway Basin they were equal at station 34, and at station 33 all of the copepods were in the upper net.

Seasonal Survey: 1984–85

We sampled five stations seven times between May 1984 and July 1985 (Fig. 4). Bad weather prevented sampling in the December–February period. Total biomass at each station was generally higher in the summer months, especially in 1985, but no clear trends were evident. This is hardly surprising in view of the high variance to mean ratios shown during geographical sampling which suggest very patchy distribution of BBL macrofauna.

Species diversity and density of amphipods and copepods were distributed seasonally as shown in Table 5. Values of K for these data are similar to those previously given with relatively high K for copepods and lower K for amphipods and other suprabenthic taxa.

Discussion

The classification of BBL macrofauna taken in the BBL sled sampler used herein is suprabenthos, zooplankton, invertebrate and vertebrate eggs and larvae, and drift epi- and infauna. Suprabenthos are bottom-dependent animals which perform daily or seasonal vertical migrations above the bottom (Sainte-Marie and Brunel 1985). In our samples, they included some cumaceans, tanaids, isopods, amphipods, mysids, and decapods. They can readily be separated from drift epi- and infauna which are incidental macrofauna dislodged from the sediment and passively drifting within the BBL.

The classification of prey organisms found in the gut of haddock larvae and juveniles during 1982–83 sampling on the continental shelf off eastern Canada (Mahon and Neilson 1987) — pelagic, benthic, and intermediate — is not supported by our results. Thus all the "pelagic" species of Appendix 1 in Mahon

[1]The complete list of the taxa identified is available at nominal cost from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ont. K1A 0S2, Canada.

TABLE 4. Characteristics of selected stations, Browns Bank, June–July 1985.

	Sediment	Station No.	Depth (m)	Gammaridea present	Gammaridea (no./m^3) Upper net	Gammaridea (no./m^3) Lower net	Copepoda (no./m^3) Upper net	Copepoda (no./m^3) Lower net
Browns Bank	Sable Island sand, <50% gravel	11	64	*Pleustes panoplus, Metopa alderi, M. bruzelli, M. pusilla, M. invalida, Anonyx* sp., *Photis reinhardi, Dyopedos monocantha, Stenula peltata, Monoculodes edwardsii, Lafystius stuionis, Ischyrocerus* sp., *Dulichia falcata*	0.21	2.71	16.94	34.59
		12	86	*Parathemisto gaudichaudi, Rhacotropis oculata, Metopa bruzelli, M. pusilla*	0.01	0.04	6.60	8.20
Fundian Channel	Sambro sand, <10% gravel	14	196	*Parathemisto gaudichaudi, Tryphosa* sp., *Ischyrocerus* sp.	0.01	0.01	8.84	11.24
		15	240	*Parathemisto gaudichaudi, Tryphosa* sp., Stenothoidae unid., *Gitanopsis bispinosa*	0.01	0.03	1.41	11.06
		33	150	*Rhacotropis distincta, Monoculodes edwardsii, Metopella* sp., *Synchelidium tenuimanum, Orchomenella pinguis, Ischyrocerus* sp.	0.01	0.01	9.08	0
Roseway Basin	Emerald silt, poorly sorted clay and sandy silt	34	168	*Haliragoides inermis, Rhacotropis distincta, Stenopleustes inermis, Metopella augusta, Argissa hamatipes, Monoculodes edwardsii, Synchelidium tenuimanum, Phoxocephalus holbolli, Ampelisca* sp., *Anonyx sarsi, Hippomedon denticulatus, Orchomenella pinguis, O. groenlandica, Tmetonyx cicada, Unciola irrorata, Erichthonius rubricornis, Ischyrocerus* sp., *Dyopedos monocanthus, Dulichia* sp., *Paradulichia typica*	0	0.31	2.76	2.96

TABLE 5. Seasonal occurrence of gammaridean amphipods and copepods as species found (*S*) and average density (*D*, no./m^3) for the stated area based on *N* net samples. Data based on five Browns Bank stations sampled during 1984–85.

			March–May			June–August			September–November		
Area	Taxa	Nets	*N*	*S*	*D*	*N*	*S*	*D*	*N*	*S*	*D*
Bank stations 6, 7, 8	Gammaridea	Upper	4	15	0.15	7	13	0.32	2	10	0.32
		Lower	4	31	5.44	8	24	0.45	1	3	0.76
	Copepoda	Upper	4	14	43.33	7	16	7.08	2	12	2.88
		Lower	4	15	15.52	8	17	4.05	1	4	4.17
Bank edge stations 20, 28	Gammaridea	Upper	2	11	0.03	2	—	0	2	1	<0.01
		Lower	3	23	0.67	2	9	0.08	1	9	<0.01
	Copepoda	Upper	2	11	56.82	2	4	34.88	2	4	0.13
		Lower	3	17	14.54	2	8	19.48	1	4	0.07

and Neilson (1987) we have commonly found within the benthic boundary layer, inclusive of hyperids, chaetognaths, and copepods, with the single exception of *Paracalanus parvus*. Pteropods also appear to be truly pelagic. Because of lack of data, it is not clear to what level in the water column benthic and intermediate taxa may reach. Hence, species identification cannot be used to decide unambiguously where in the water column the prey item was obtained. This probably does not change the conclusion of Mahon and Neilson (1987) regarding the timing of juvenile haddock vertical migrations because most of the amphipods and polychaetes found in juvenile stomachs were truly benthic.

Like other benthic devices which must sample in an extremely patchy environment, the variance to mean ratios of repeated geographic and temporal samples are large, rendering direct statistical comparisons problematical. Additional confounding factors, besides the patchy distribution of BBL macrofauna, include net avoidance by the larger swimming macrofauna and a possible effect of tidal flow on sampling efficiency (Stephenson and Sochasky 1990) which was not recognized during our study. Finally, the difficulty of accurately positioning the sled remotely from the towing vessel so that sampling occurs on only one sediment type further complicates the sampling problems involved with the BBL sled. Despite these difficulties, it has been shown by Sainte-Marie and Brunel (1985) that some suprabenthic animals swim at characteristic levels within, or just above, the BBL. Our results with some species of cumaceans and amphipods, for which we have sufficient replicates, confirm that significantly more individuals are present at the level of the lower net.

The diet of age-0 juvenile haddock changes as they grow: from an initial preponderance of smaller copepods in June (69% of the total number of diet items) to 4.7% in October (Mahon and Neilson 1987). This is complemented by an increase in size of the prey items which are consumed such as polychaetes and amphipods (maximum size of 15–30 mm). Consideration of

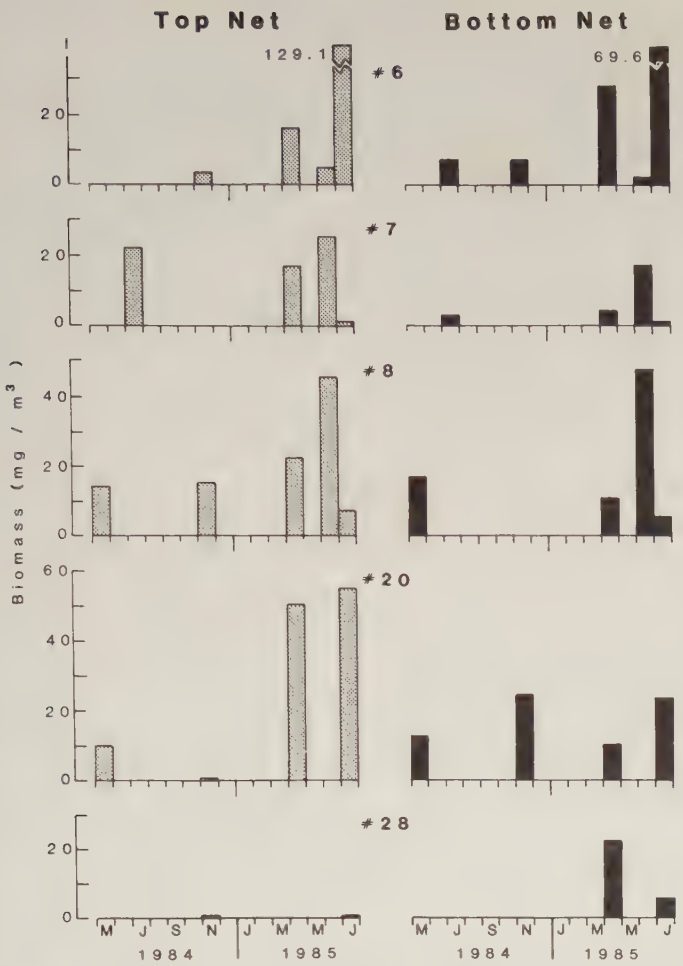

FIG. 4. Seasonal distribution in 1984–85 of BBL mean macrofaunal biomass in the two nets.

Table 2 suggests that the haddock juvenile can choose a wide range of sizes, from invertebrate eggs 0.4 mm in diameter to the decapod *Pandalus* of over 100 mm in body length. However, gut content analyses (Mahon and Neilson 1987) suggest that the most available potential prey within the benthic boundary layer are not eaten by demersal juvenile haddock (>10 cm in body length): the most important prey items in October included the ampharetid polychaete *Melinna cristata*; the aorid amphipods *Unciola* sp., and *Leptocheirus pinguis*; ampeliscid and caprellid amphipods. Neither *M. cristata* nor *L. pinguis* were found in our BBL sled catches and the other prey items were uncommon (i.e. not included in Table 2). *Unciola irrorata* was a common and productive species in Browns Bank grab samples (Wildish et al. 1989). It was also occasionally taken in the BBL sled samples (Table 4). *Melinna cristata* and *L. pinguis* were less common but taken in many grab hauls (D. J. Wildish, unpublished). The prey animals absent from BBL sled catches may have been missed because it sampled too far above the sediment (>33 cm). This suggestion is consistent with the hypothesis (Wildish et al. 1989) that haddock juveniles prey upon epi- and infauna that are occasionally swept away by the generally energetic tidal flows on banks. Another possibility consistent with the data is that the prey are cropped by juvenile haddock directly from the sediment. The latter appears to be less likely because prey (amphipods, polychaetes) are well camouflaged against the sedimentary background (D. Wildish, unpublished underwater photographs) and juvenile haddock lack obvious specializations (eye position, chemosensory or tactile structures) for bottom feeding.

Just after transition from pelagic to demersal life, juvenile haddock become associated with pebble/gravel deposits on Georges Bank and are absent on rippled sand substrates (Lough et al. 1989). This behavior was suggested to involve predator avoidance (juveniles well camouflaged on gravel deposits) and, to a lesser extent, increased abundances of prey organisms on this substrate. Lough et al. (1989) also outlined a diurnal cycle of behavior: close to the bottom (a few centimeters) during the day and in the near-bottom (a few metres) water column at night; they directly observed the importance of flow velocity in this behavior. By remaining close to the bottom during high flow velocities, fish would minimize energy expenditure due to the logarithmic decrease of velocity towards the bottom. We suggest that this behavior is regulated by cyclical tidal velocities as well as diurnal cycles and that feeding occurs mostly during the day within a few centimetres of the bottom. This is consistent with gut content analysis (Mahon and Neilson 1987), the hypothesis that haddock are visual predators (Brunel 1968; Mahon and Neilson 1987), and that younger, pelagic juveniles are diurnal feeders (Perry and Neilson 1988). We suggest that during the day, like salmonids in a stream, juvenile haddock swim into the current and as close to the bottom as possible to conserve energy while they await drifting macrofauna to come into their field of vision. During daytime slack currents, they may disperse within the BBL.

Both our field observations and those of other workers have suggested causal hypotheses which are ripe for experimental testing. In particular, the hypothesis that juvenile haddock are feeding on drift macrofauna versus cropping epi- or infauna could be experimentally tested by studies in a laboratory flume.

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Circannual Cycle of Seawater Adaptability in *Oncorhynchus nerka*: Genetic Differences between Sympatric Sockeye Salmon and Kokanee

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Anadromous sockeye salmon and nonanadromous kokanee (*Oncorhynchus nerka*) spawn sympatrically in locations across British Columbia, yet remain genetically distinct. To investigate the possibility that these differences are maintained by selection against hybrids of the two forms owing to differences in seawater adaptability, we raised pure and reciprocal crosses of Shuswap River sockeye and kokanee in controlled hatchery conditions and tested their seawater adaptabilities nine times over 2 yr. Sockeye, kokanee, and reciprocal hybrids all showed a seasonal cycle in their seawater adaptability, which was lowest in midwinter and highest in the spring. However, clear differences were evident among cross-types in the spring of their second year. Sockeye were the first to show increased seawater adaptabilities, hybrid groups next, and kokanee last. The observed differences could not be attributed to tank effects, or to differences in size, sex, or state of maturity.

Le saumon nerka anadrome et sa forme non anadrome, le kokani (*Oncorhynchus nerka*), fraient de façon sympatrique en plusieurs lieux de la Colombie-Britannique, mais demeurent génétiquement distincts. Afin d'étudier la possibilité que ces différences soient maintenues par une sélection s'exerçant à l'encontre des hybrides des deux formes, pour des raisons d'adaptation à l'eau de mer, nous avons élevé en pisciculture et dans des conditions contrôlées des produits de croisements purs et réciproques de saumons nerkas et kokanis de la rivière Shuswap et vérifié leurs capacités d'adaptation à l'eau de mer à neuf reprises au cours d'une période de 2 ans. Les saumons nerkas et kokanis de même que leurs hybrides réciproques ont tous présenté un cycle saisonnier d'adaptation à l'eau de mer qui était le moins élevé au milieu de l'hiver et le plus élevé au printemps. Des différences nettes étaient cependant évidentes entre les poissons de types de croisement différents au cours de la deuxième année. Les nerkas ont été les premiers à présenter une capacité accrue d'adaptation à l'eau de mer, venaient ensuite les hybrides et, en dernier, les kokanis. Les écarts observés ne pouvaient être attribués à des effets liés aux cuves ou à des différences de taille, de sexe ou de stade de maturité.

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Smoltification describes a series of physiological, morphological, and behavioural changes which allows salmonids to pass from fresh water to seawater, most often in the spring of the year (see reviews by McCormick and Saunders 1987; Hoar 1988). One of these changes, the seasonal increase in hypoosmoregulatory capacity, is well studied, since it is readily measured as the ability to regulate plasma sodium concentrations during a 24-h seawater challenge test (Blackburn and Clarke 1987). The ability of juvenile salmon to regulate plasma sodium concentrations rapidly in a 24-h seawater challenge test has been shown to be correlated with the ability to survive and grow in seawater (Clarke 1982; Clarke and Shelbourn 1986). This is distinct from the ability to merely survive in 30‰ seawater which may be attained prior to the completion of smolting; smolts are able to survive in salinities of 35–40‰ (McCormick and Saunders 1987; Johnsson and Clarke 1988; Clarke et al. 1989).

The ontogeny of seawater tolerance differs among salmonid genera and species, reflecting the ecology of individual species (Rounsefell 1958; Parry 1960; Weisbart 1968; McCormick et al. 1985; McCormick and Saunders 1987). For example, pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) are able to tolerate seawater at small sizes, in accordance with their seaward migration very soon after emergence (Weisbart 1968). At the opposite end of the spectrum, brook char (*Salvelinus fontinalis*) are unable to tolerate seawater at small sizes and generally spend several years in fresh water before migrating to sea (McCormick et al. 1985). Moreover, lake char (*S. namaycush*) are unable to tolerate seawater at any stage of their life (Rounsefell 1958; McCormick and Saunders 1987).

Differences in the smoltification process also exist within and between populations of the same species. Fish may smolt at a variety of ages, depending on conditions for growth (e.g. temperature, food availability, and length of growing season; Burgner 1987), developmental characteristics of individual fish (e.g. Thorpe 1977; Bailey et al. 1980; Metcalfe et al. 1988; Metcalfe 1989), and difficulty of seawater migration (Taylor 1990).

In a series of papers, we have investigated the plausibility of sympatric genetic divergence of anadromous and nonanadromous *Oncorhynchus nerka* first proposed by Ricker (1938,

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1940). Sockeye salmon and kokanee are polyphyletic, having given rise to one another on numerous occasions (Ricker 1940; Foerster 1947; Nelson 1968; Foote et al. 1989). Where they occur and spawn sympatrically, they are genetically distinct yet appear more closely related to one another than to their corresponding form in separate watersheds (Foote et al. 1989). The possibility that this pattern has resulted from sympatric divergence is supported by the ability of one form to give rise to the other (Ricker 1959, 1972; Scott 1984), the vastly different selective regimes of the marine and lacustrine environments (Ricker 1940; Wood and Foote 1990; Taylor and Foote 1991), and because the forms mate assortatively by size (Hanson and Smith 1967; Foote 1988) and hence by type (Foote and Larkin 1988). However, while there is extensive assortative mating by form, prezygotic isolation is not complete; kokanee males sneak in on sockeye spawnings, thus creating the continual opportunity for the homogenizing effects of gene flow between forms. The resulting hybrids are fully viable under hatchery conditions (McCart 1970; Wood and Foote 1990). Therefore, the question remains: how is reproductive isolation maintained in the face of continual opportunity for gene flow between forms?

Here, we report on the results of a study of the ontogeny of seawater adaptability in pure and reciprocal crosses of sympatric sockeye salmon and kokanee. Given that smoltification is undoubtedly an adaptive response to enable fish to move from a freshwater to marine environment (Hoar 1976, 1988; McCormick and Saunders 1987) and apparently unnecessary and perhaps even disadvantageous to nonanadromous fish (e.g. Maule et al. 1987; McCormick and Saunders 1987), we hypothesized that sockeye salmon would show a marked increase in the ability to regulate plasma sodium levels and associated seawater tolerance as part of their natural smolting process whereas these developments would be lost or muted in kokanee.

Methods

Broodstock Collection and Rearing Environment

Sockeye and kokanee spawn sympatrically in the Lower Shuswap River, British Columbia, yet remain genetically distinct (Foote et al. 1989; Wood and Foote 1990). On October 12, 1986, we collected sockeye and kokanee from the spawning grounds using a small seine. We created four cross-types: pure sockeye (S♀*S♂), pure kokanee (K♀*K♂), and the reciprocal hybrids (K♀*S♂ and S♀*K♂) by combining sperm and eggs of multiple fish (36) from the appropriate sources. Each cross-type consisted of six pairs of half-sib families (12 families per each cross-type; six males each mated to two females; 48 families in total). The gametes of males and females were split between cross-types (i.e. the same males that were used to sire pure kokanee offspring were also used to sire S♀*K♂ offspring, and the same females which were used to produce pure kokanee offspring were used to produce K♀*S♂ offspring). A detailed description of the genetic design is given in Wood and Foote (1990).

The fertilized eggs were immediately transported by truck to the Rosewall Creek Hatchery (RCr) on Vancouver Island, where they were incubated at approximately 8.5°C, until February 4, 1987. Thereafter, the fry were raised by family in individual 480-L tanks, with flowing 8.5°C well water. The free-swimming fry were fed Oregon Moist Pellets (of appropriate size) ad libitum dispensed by automatic feeders every 30 min during daylight on a natural photoperiod. Each of the 48 families was culled randomly to a maximum of 300 fry before ponding on February 4 and this number was further culled randomly to a maximum of 130 per tank 99 d later. In addition to these 48 individual families, we maintained four 480-L tanks consisting of pooled groups of families of pure sockeye (PS), pure kokanee (PK), and the reciprocal hybrids ("hybrid sockeye," HS; sockeye females by kokanee males and "hybrid kokanee," HK; kokanee females by sockeye males). Each group consisted of pooled families of surplus fish from the above 48 families and those from 24 additional unused families taken from the same site at the same time (see Wood and Foote 1990). These fish were reared at densities of over 500 fry per tank until mid-July, when all were culled randomly to approximately 300 fish per tank.

The fish that were raised by family as underyearlings were used to examine the ontogeny of seawater adaptability as yearlings, the normal smolting year for Shuswap Lake sockeye. The availability of surplus fish allowed us to examine the ontogeny of seawater adaptability in underyearlings as well, and the combination of the two groups permitted us to examine the circannual cycle in seawater adaptability over the first 21 mo past swim-up. However, this combination was complicated by the fact that the surplus groups were raised at higher densities than those used for yearling analysis. To determine the extent of the density effect on seawater adaptability, we compared all cross-types from both groups at the same time in January 1988. Given that the two groups were raised at different densities and hence experienced different growth conditions, we refer to the pooled groups of underyearling fry as the "high-density treatment" and the groups originally raised as individual families for testing as yearlings as the "low-density treatment."

The high-density underyearling treatment groups were maintained until late January 1988, at which point the remaining fish were killed by anaesthesia. The low-density yearling groups were maintained in individual tanks until late January 1988 at which point they were pooled by cross-type into four large (1800-L) "single cross-type" tanks. Each single cross-type tank contained 10 (PK), 11 (PS and HK), or 12 (HS) families and each family comprised 30–40 individuals to make a total of approximately 400 fish per tank. Coded wire nose tags were used to identify fish as to family (see Bilton et al. 1982).

To control for possible tank effects on seawater adaptability, two additional 1800-L "mixed cross-type" tanks of the low-density yearling treatment groups were maintained. Owing to space and sometimes family limitations, only one half of the genetic design outlined above was used. One tank contained fish from five PK families (adipose fin clipped) and six HS families (no clip; three kokanee males mated to two kokanee and two sockeye females each; one PK family missing) and the other contained fish from five PS (adipose clipped) and six HK families (no clip; three sockeye males mated to two kokanee and sockeye females each; one PS family missing). As before, 30–40 fish from each family were kept to make a total of approximately 400 fish per tank. Again, each fish was nose tagged to identify family of origin.

The four single cross-type tanks and the two mixed cross-type tanks were maintained in the same building, on the same well-water source (8.5°C), and on a simulated natural photoperiod until late March 1988, when the fish of the mixed cross-type tanks were transferred to similar sized tanks at the Pacific Biological Station (PBS) in Nanaimo. These fish were maintained on dechlorinated Nanaimo city water until June and

experienced the same photoperiod. However, at PBS the water temperature increased from 9 to 12°C over the test period.

Seawater Adaptability Tests

The seawater adaptability of each cross-type was measured by 24-h seawater challenge tests at PBS on nine occasions from 4 to 21 mo after swim-up (see Blackburn and Clarke 1987 for challenge test details). For each test, 20–45 fish of each cross-type were transported in separate aerated tubs from RCr to PBS at least 4 d before testing, where they were maintained in separate tanks of dechlorinated Nanaimo city water (8.5–9.0°C). After 4 d, the fresh water was displaced by natural seawater (29–30‰) of controlled temperature (8.5–9.0°C). After 24 h in seawater, each fish was killed by anaesthesia (500 ppm 2-phenoxyethanol) and a blood sample taken from the caudal artery. This sample was analyzed for plasma sodium concentration using a Turner (Case Instruments) flame photometer. After challenge, fish that have acquired the hypoosmoregulatory capacity for living in seawater typically show plasma sodium levels lower than 170 mmol/L whereas fish that have not completely adapted to seawater suffer substantial increases in plasma sodium levels (Clarke 1982; Boyce and Clarke 1983; Blackburn and Clarke 1987). Immediately before the blood sample was taken, each fish was measured for fork length to the nearest 1 mm and weighed to the nearest 0.1 g.

To test for possible effects of sex and maturation on seawater adapatability, the sex and state of gonadal development of yearling fish were noted in the May, June, August, and October samples of 1988.

Underyearling fish from the high-density treatment were tested on June 10 and October 23, 1987, and again on January 21, 1988. Fish from the low-density treatment were tested on January 21, March 28, April 29, May 31, June 30, August 9, and October 18, 1988. Fish from both treatments were selected randomly from pooled tanks. In May 1988, we sampled yearling fish from both PBS and RCr to test for possible tank (and location) effects on seawater adaptability.

Salinity Tolerance Tests

We also tested the ability of yearling sockeye, kokanee, and the reciprocal hybrids to tolerate two hypersaline conditions (39 and 41‰) in early June 1988. Saunders and Henderson (1970, 1978) reported that challenging juvenile Atlantic salmon (*Salmo salar*) to high salinity (35–40‰) is an effective means of demonstrating additional salinity tolerance that is undeveloped before smoltification. General methods for this procedure are described in Saunders et al. (1985) and Blackburn and Clarke (1987). In short, we created baths of 39 and 41% in four 200-L tanks each using natural seawater mixed with Hagen Marine Mix Biocrystals. We used these relatively high salinities because the seawater adaptability tests of late May 1988 revealed that all forms showed at least some of the increased hypoosmoregulatory ability associated with smoltification. These tanks were aerated and maintained on the natural photoperiod with the temperature maintained between 8 and 9°C. Into each of a pair of 39 and 41‰ tanks, we placed 20 fish of each cross-type and monitored their survival at 24-h intervals over 4 d.

Statistical Analyses

Because the variance of plasma sodium levels often differed significantly among cross-types ($P < 0.05$, Bartlett's test), all sodium measurements were transformed to $Z_i = \log(X_i - 152)$ prior to analysis, where X_i is the sodium level (millimoles per litre) of the ith fish. Where there was a significant relationship between fork length and transformed plasma sodium, transformed sodium values for individual fish were further adjusted to a standardized length using the relationship $Z_i' = Z_i + b(L_i - L)$ where b is the slope of the regression of transformed plasma sodium (Z_i) on fork length (L_i) and L is the mean fork length of the pooled sample. The common slope for all cross-types combined was used where there was no significant difference in slope across groups (ANCOVA). Otherwise, individual slopes were used for each cross-type.

Transformed sodium levels for the high- and low-density treatments were analyzed separately (with the exception of the January 1988 samples from both treatments). A three-way fixed ANOVA was used for each treatment, with the main effects being date of challenge, female type (sockeye or kokanee), and male type. Within treatments, within sample periods (date), sodium levels were analyzed using a two-way fixed ANOVA model, with the main effects being male type and female type.

We first compared the seawater adaptability of the different forms after pooling fish from different families according to cross-type (Analysis A). Strictly speaking, these results apply only to the tank populations and not to those in the wild because family effects were not considered explicitly. To avoid pseudoreplication and yet address statistically the question of genetic differentiation of the forms in the wild, we also identified with the use of the nose tags the family of origin for each fish sampled in March, April, June, and August 1988 and analyzed the results at the family level. Only families from which at least two fish were tested were considered in the analyses. It was not possible to use the full ANOVA model employing the nested design within cross-types because of the large number of empty or nonreplicated cells. Instead, independent (full-sib) family means were compared by two-way fixed ANOVA for each sample period (Analysis B). In this analysis, the number of data points in each cell equalled the number of independent parents sampled in the wild (i.e. a maximum of six full-sib families per cross-type for fish held at RCr and a maximum of three families per cross-type for those held at PBS). Given that two females were mated to the same male in each case, only one of these families could be used to insure independence in this rigorous but conservative analysis. We selected the family with the larger number of progeny sampled, or selected randomly where sample sizes were equal.

To investigate differences among families within cross-type, we compared full sib families within cross-types in seawater adaptability (ANOVA).

Results

Growth rates were similar among cross-types for both the underyearling high-density groups and the yearling low-density groups (Tables 1 and 2; see Wood and Foote (1990) for a detailed examination of growth in Shuswap River sockeye and kokanee). Within treatments, significant differences ($P < 0.05$, ANOVA) in fork length among cross-types were evident only in the January 1988 high-density treatment and in the October 1988 low-density treatment. Significant differences in weight among cross-types were observed in the June and January high-density treatment ($P < 0.01$) and in the January ($P < 0.05$) and October ($P < 0.001$) low-density treatment. Fish in the low-density groups grew faster than those in

TABLE 1. Mean fork length (FL), weight (WT) (±SE), plasma sodium concentration ([Na]), and the correlation between fork length and plasma sodium concentrations for each of three sample periods for pure sockeye (PS), pure kokanee (PK), and their reciprocal hybrids (HS, HK; hybrid group named after the female parent type) when reared at high density. Significance levels and proportion of variance accounted for by the main effects of two-way ANOVA of female type (FT) and male type (MT) on plasma sodium concentration are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; (*)$P < 0.10$; ns = nonsignificant).

							ANOVA		
Date	Type	FL (cm)	Wt (g)	[Na] (mmol/L)	N	r	FT	MT	FT × MT
June 10, 1987	PK	7.0 ± 0.1	2.57 ± 0.11	167.3 ± 1.3	36	−0.55***			
	HK	6.8 ± 0.1	2.34 ± 0.10	162.6 ± 1.0	36	0.04	**	ns	***
	HS	7.1 ± 0.1	2.76 ± 0.08	159.5 ± 0.7	36	−0.47**	5.7%	0.1%	7.3%
	PS	7.0 ± 0.1	2.57 ± 0.11	163.8 ± 1.0	36	−0.57***			
Oct. 23, 1987	PK	9.8 ± 0.3	8.64 ± 0.76	178.0 ± 1.3	27	−0.37*			
	HK	10.5 ± 0.3	10.55 ± 0.89	172.7 ± 1.7	26	−0.24	ns	ns	***
	HS	10.3 ± 0.2	9.57 ± 0.53	175.6 ± 1.2	30	−0.07	1.4%	0.8%	9.8%
	PS	10.1 ± 0.3	9.22 ± 0.85	179.1 ± 1.5	27	−0.50**			
Jan. 21, 1988	PK	11.2 ± 0.3	13.15 ± 1.08	184.9 ± 1.3	30	−0.24			
	HK	12.2 ± 0.3	17.38 ± 1.10	180.1 ± 1.1	30	−0.06	ns	ns	(*)
	HS	12.0 ± 0.2	16.35 ± 1.12	183.0 ± 1.5	29	−0.08	0.0%	1.8%	3.1%
	PS	11.1 ± 0.3	12.80 ± 1.03	183.5 ± 1.6	30	0.10			

the high-density groups ($P < 0.001$; comparison of January 1988 high- and low-density treatments). In most cases there was a negative relationship between plasma sodium concentration and fork length; larger fish were more tolerant of seawater (Tables 1 and 2).

High-Density Treatment (Underyearlings)

All groups displayed "full" seawater adaptability (plasma sodium concentrations less than 170 mmol/L) in the spring of their first year, but their ability to tolerate seawater declined steadily thereafter ($P < 0.001$; Table 1). Neither female nor male type alone had any significant effect on seawater adaptability in the three-way model ($P > 0.15$), but a significant female type – male type interaction was detected ($P < 0.001$); both reciprocal hybrid groups exhibited lower blood plasma sodium concentrations than the two pure groups. This interaction term was significant in both the June and October sample periods ($P < 0.001$) but not in January ($P < 0.10$).

Low-Density Treatment (Yearlings)

There was a general pattern for all groups to display low seawater adaptability (>170 mmol/L) during the winter, for seawater adaptability to increase during the spring to early summer, and to decrease thereafter (date effect: $P < 0.001$; Fig. 1). In addition, male and female type effects, female type – male type interactions, and the date – female type – male type interactions were all significant in the three-way model ($P < 0.001$).

Pure sockeye showed evidence of increased seawater adaptability by late March in 1988 whereas pure kokanee did not display increased seawater adaptability until late May. The timing of increased seawater adaptability in hybrid sockeye and hybrid kokanee was intermediate to the pure forms; an improvement in seawater adaptability first became apparent in late April. Sockeye first attained full adapatability to seawater before the end of April, the hybrid forms by late May, but kokanee not until late June.

There were also significant female type and male type effects on seawater adaptability in the two-way model (within sampling periods) in the March, April, May, August, and October samples (Table 2). In all cases, kokanee displayed the lowest ability to regulate plasma sodium ion levels whereas sockeye displayed the highest. The interaction of male and female type effects was significant only in the April sample ($P < 0.05$) but was marginally significant ($P < 0.10$) in the January, March, May (PBS), and August samples. There were no significant differences in the late June sample, by which time all cross-types showed full adaptability to seawater.

A total of six fish died during 24 h of exposure to seawater, excluding the October 1988 sample (see later). Three kokanee and one hybrid sockeye died during the April 1988 test and two hybrid sockeye died during the May 1988 test.

Comparison of High- and Low-Density Treatments (January 1988)

Rearing density had an effect ($P < 0.001$) on seawater adaptability in the three-way ANOVA model (male type, female type, and density treatment). However, there were no significant interactions of density treatment and male and female cross-types ($P > 0.2$). This indicates that the effects of density treatment were similar for all cross-types. Male type and female type effects were both nonsignificant whereas there was an interaction effect ($P < 0.01$).

The density effect did not result simply from the size difference between treatments. The effect of rearing density was still significant ($P < 0.001$) when all data were adjusted to the overall sample mean fork length (13.06 cm) using the common slope (−0.068). Within every cross-type, fish reared at low densities displayed greater adaptability to seawater both before and after size adjustment, although the difference between treatments was not significant in pure sockeye ($P > 0.05$). It is important to note that while rearing density had a significant effect on seawater adaptability, this effect was relatively minor. The ability of fish to regulate plasma sodium was relatively low in both density treatments and in all cross-types.

Salinity Tolerance Tests

Consistent with the seawater adaptability tests in late May 1988, sockeye displayed the highest survival rate at 39 and 41‰, kokanee showed the lowest survival rates, and the hybrids

TABLE 2. Mean fork length (FL), weight (Wt) (± SE), and the correlation between fork length and plasma sodium concentration for each of seven sample periods for yearling pure sockeye (PS), pure kokanee (PK), and their reciprocal hybrids (HS, HK; hybrid group named after the female parent type). Significance levels and proportion of variance accounted for by the main effects of two-way ANOVA of female type (FT) and male type (MT) on plasma sodium concentrations are also shown (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; (*)$P < 0.10$; ns = nonsignificant). Analysis A uses all family data pooled within cross-type; Analysis B uses only independent (full-sib) family means (see Methods). Significance levels for Analysis A using only nonmaturing fish are given in square brackets.

						Analysis A			Analysis B		
Date	Type	FL (cm)	Wt (g)	N	r	FT	MT	FT × MT	FT	MT	FT × MT
Jan. 21,	PK	13.9 ± 0.2	26.5 ± 1.5	30	−0.70***						
1988,	HK	14.9 ± 0.2	33.0 ± 1.7	35	−0.64***	ns	ns	(*)			
RCr	HS	14.3 ± 0.2	28.8 ± 1.3	30	−0.41**	0%	0%	3%			
	PS	14.6 ± 0.3	30.8 ± 1.7	36	−0.50***						
Mar. 28,	PK	14.9 ± 0.4	32.8 ± 3.1	36	−0.63***						
1988,	HK	15.9 ± 0.3	40.7 ± 2.8	36	−0.59***	**	**	(*)	*	*	ns
RCr	HS	15.6 ± 0.2	37.9 ± 1.9	36	−0.22	6%	6%	2%	16%	22%	6%
	PS	15.7 ± 0.2	38.5 ± 1.7	36	−0.35*						
Apr. 29,	PK	16.2 ± 0.4	37.7 ± 3.2	30	−0.76***						
1988,	HK	16.6 ± 0.3	45.3 ± 2.8	29	−0.34(*)	***	***	*	***	***	ns
PBS	HS	17.0 ± 0.3	46.6 ± 2.5	30	−0.55***	19%	37%	2%	28%	62%	1%
	PS	16.7 ± 0.4	45.8 ± 3.3	26	−0.53**						
May 31,	PK	17.8 ± 0.5	54.9 ± 4.8	20	−0.33						
1988,	HK	17.8 ± 0.5	54.9 ± 5.0	19	0.35	***	**	ns			
RCr	HS	17.9 ± 0.4	56.6 ± 4.7	20	0.45*	28%	7%	0%			
	PS	17.5 ± 0.4	51.8 ± 3.7	19	−0.05	[***]	[**]	[ns]			
May 31,	PK	17.5 ± 0.5	53.9 ± 4.7	25	−0.45*						
1988,	HK	17.9 ± 0.4	57.9 ± 4.1	26	0.07	***	***	(*)			
PBS	HS	18.2 ± 0.4	62.7 ± 4.8	27	−0.33(*)	15%	15%	3%			
	PS	18.0 ± 0.2	57.8 ± 2.4	24	−0.30	[***]	[***]	[(*)]			
June 30,	PK	17.6 ± 0.6	57.5 ± 6.1	24	0.03						
1988,	HK	18.6 ± 0.4	66.7 ± 4.6	39	0.32	ns	ns	ns	ns	ns	ns
RCr	HS	18.0 ± 0.3	59.4 ± 3.2	42	0.06	1%	0%	0%	2%	0%	1%
	PS	17.9 ± 0.4	57.8 ± 3.6	28	0.31	[ns]	[ns]	[ns]			
Aug. 8,	PK	20.3 ± 0.6	88.3 ± 8.6	24	−0.66***						
1988,	HK	19.7 ± 0.4	78.5 ± 6.3	38	−0.17	***	***	(*)	***	*	ns
RCr	HS	19.9 ± 0.3	77.0 ± 4.5	41	0.23	32%	6%	1%	64%	9%	2%
	PS	18.7 ± 0.4	63.5 ± 4.1	25	0.36(*)	[***]	[*]	[ns]			
Oct. 18,	PK	24.6 ± 0.7	170.7 ± 13.2	24	0.19						
1988,	HK	22.8 ± 0.8	146.0 ± 14.3	22	0.35(*)	***	***	ns			
RCr	HS	22.1 ± 0.4	112.3 ± 7.4	20	0.01	23%	23%	1%			
	PS	22.0 ± 0.4	116.3 ± 7.6	24	−0.62***	[(*)]	[*]	[*]			

displayed intermediate tolerance levels when tested in early June (Fig. 2; $P < 0.01$ in both cases, χ^2 likelihood ratio).

Examination of Possible Tank (and Location) Effects

Rearing location had a slight, but significant, effect on seawater adaptability in comparisons among yearlings in May 1988 ($P < 0.05$; Table 2). In a three-way model (female type, male type, and rearing location), rearing location accounted for only 1.9% of the variation in seawater adaptability compared with 31.2% of the variation accounted for by female and male type effects combined. Within cross-types, only pure sockeye showed a difference between the two rearing locations (161.4 ± 1.0 (SE) mmol/L RCr vs. 165.5 ± 1.1 PBS; $P < 0.01$). However, sockeye were fully adapted to seawater in both locations and the relative order of seawater adaptability among cross-types remained unchanged in the two rearing locations; sockeye were the best adapted to seawater, followed by the reciprocal hybrid groups (HS: 165.7 ± 1.5 RCr vs. 167.9 ± 1.2 PBS; HK: 171.9 ± 2.4 RCr vs. 168.7 ± 1.5 PBS) and then kokanee (177.6 ± 3.1 RCr vs. 179.1 ± 2.2 PBS).

Effect of Sex and State of Maturity on Seawater Adaptability

There were significant differences among cross-types in the proportion of individuals maturing as yearlings ($P < 0.001$, χ^2 likelihood ratio). Within males, kokanee showed the highest tendency to mature in their second year (78.6%, 55 of 70; maturity data pooled for May, June, August, and October samples) and sockeye the lowest (3.6%, 2 of 55), with the reciprocal hybrids roughly intermediate (HK: 24.7%, 19 of 77; HS: 31.1%, 28 of 90). Maturing females were rare to absent in all cross-types but kokanee (36.7%, 18 of 49). No individuals matured as underyearlings.

In the May, June, and August samples, neither individual sex nor state of maturity had detectable effects on seawater adaptability within any group (four-way ANOVA: female type, male type, sex, and state of maturity, $P > 0.10$; Fig. 3). In contrast, there were differences between the sexes in seawater adaptability in the October sample ($P < 0.005$), the natural spawning time of the tested populations. This resulted from the reduced seawater adaptability of all mature males in all groups

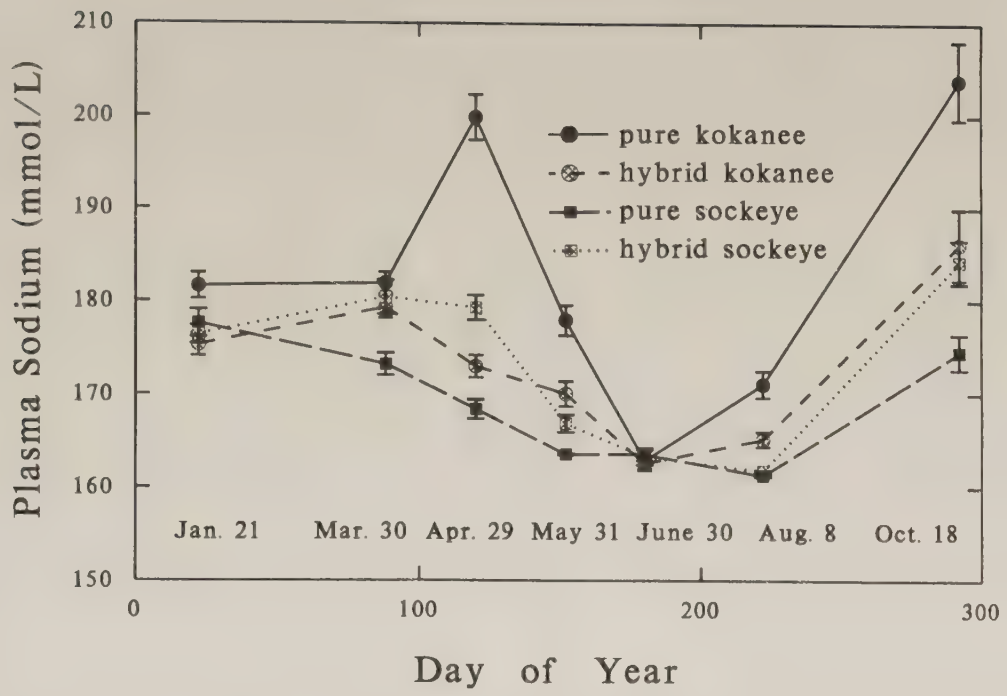

FIG. 1. Seasonal change in the mean plasma sodium concentrations ± SE (seawater adaptability) of yearling sockeye, kokanee, and their reciprocal hybrids (hybrid type named after the female parent) after 24 h of exposure to natural seawater (29–30‰). Note that these data required log transformation prior to ANOVA.

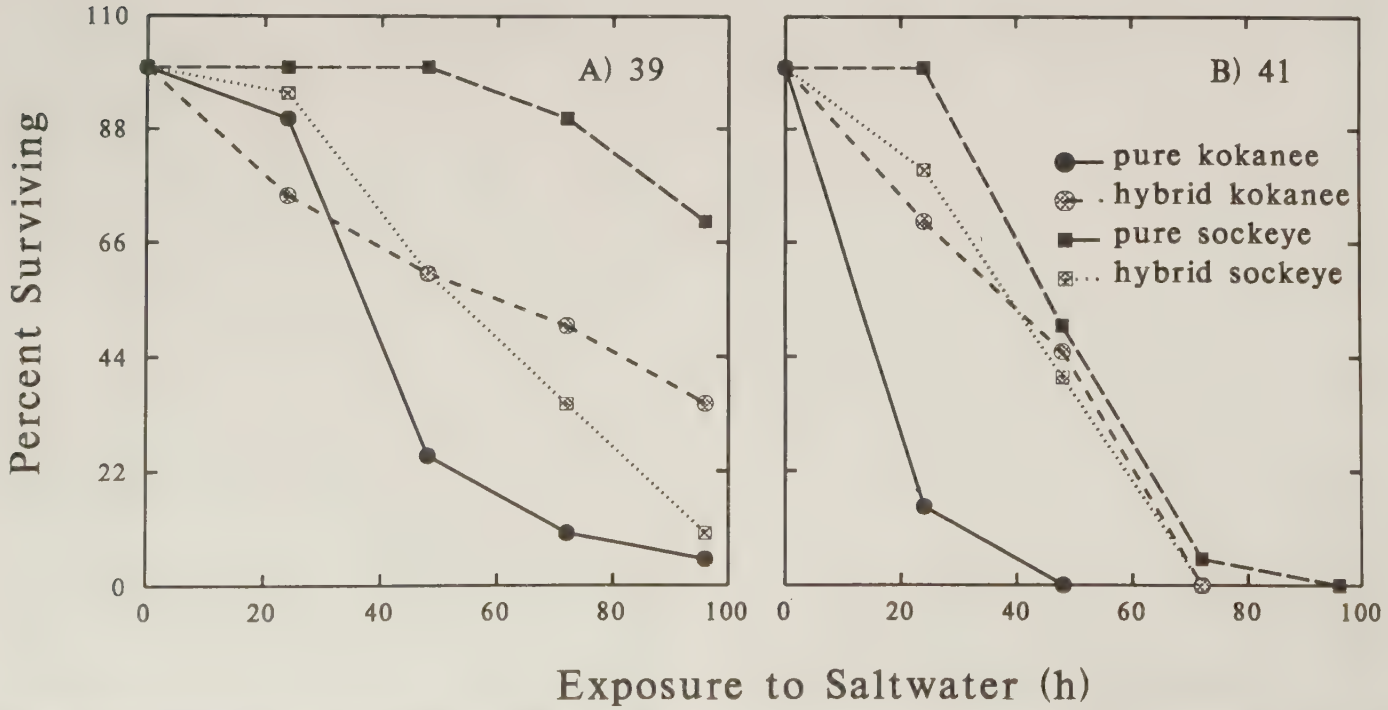

FIG. 2. Saltwater tolerance of yearling sockeye, kokanee, and their reciprocal hybrids (hybrid type named after the female parent) over 4 d of exposure to (A) 39‰ and (B) 41‰ salt water in early June.

(i.e. sex-linked differences in maturity confounded the analysis). Five mature males (two PK, two HK, one HS) but no others died over the 24-h exposure to seawater. In contrast with mature males, mature female kokanee were as tolerant of seawater as were nonmature kokanee ($P > 0.75$, t-test).

Analyses using only nonmaturing fish for the months of May, June and August produced near identical results to those using the total sample (Table 2). Analysis of only nonmaturing fish in the October sample produced significant ($P < 0.05$) or marginally significant ($P < 0.10$) male and female type effects,

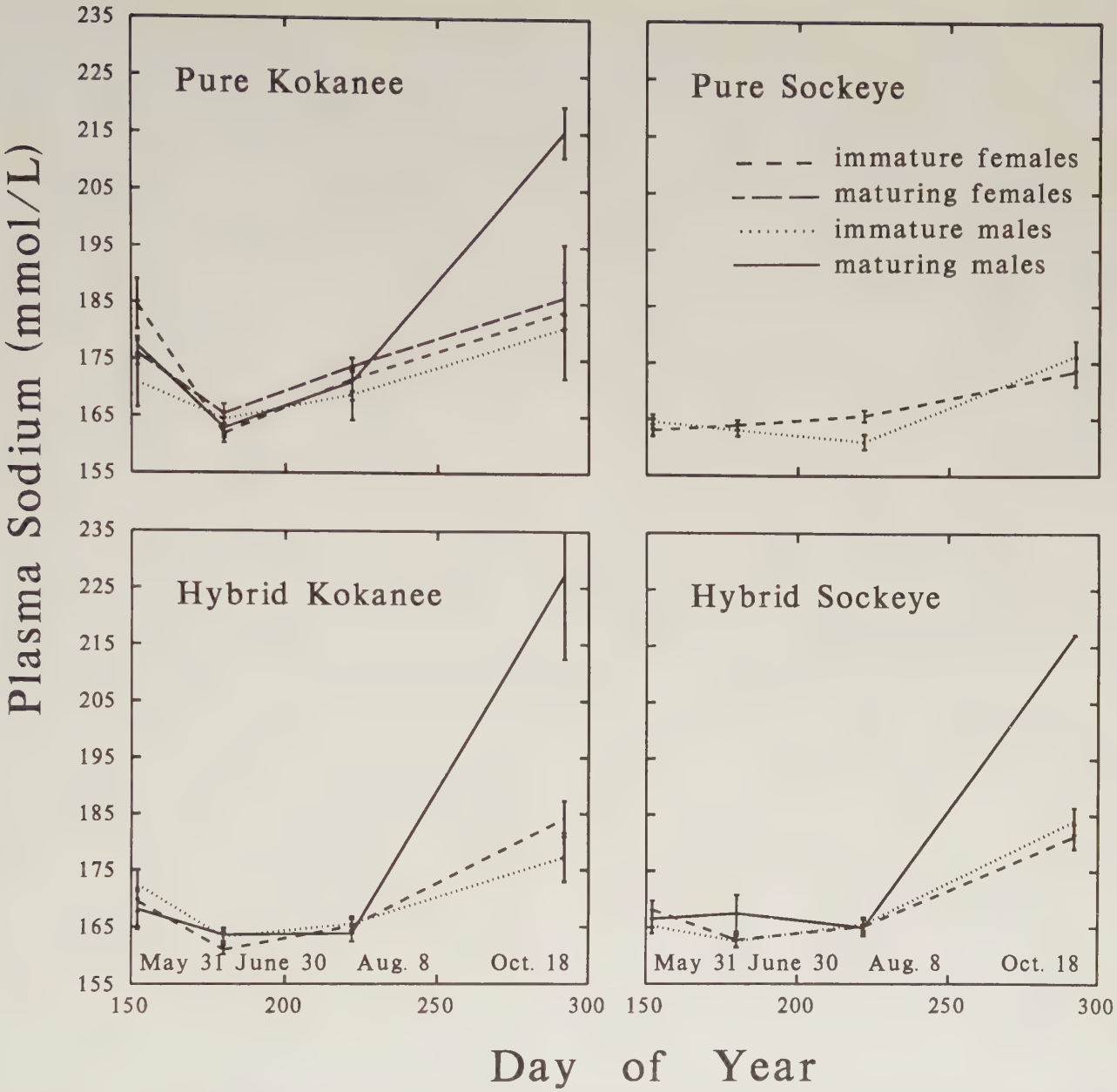

FIG. 3. Mean plasma sodium concentrations ± SE of immature and maturing yearling male and female kokanee, sockeye, and their reciprocal hybrids (hybrid type named after the female parent) after 24 h of exposure to natural seawater (29–30‰) during the May, June, August, and October sampling periods. Note that these data required log transformation prior to ANOVA.

but in this case the effects were much smaller than in the total sample.

There were sufficient samples of maturing and nonmaturing kokanee males in the months of May, June, and August to examine the manner in which maturing and nonmaturing individuals partitioned their growth into length versus weight increments (ANCOVA). In other salmonids, precocious males typically gain more weight per increment of length than nonmaturing individuals (e.g. Rowe and Thorpe 1990; Foote et al. 1991). In all months, there were no differences between maturation types in the regression of the log fork length on log weight (slope and elevation, $P > 0.20$; $R^2 > 0.98$ for all cases). In addition, we noted no difference in colour between maturing and nonmaturing individuals in May, June, and August; all fish examined were silver.

Variation among Families within Cross-Types

We looked for significant differences among full-sib families within cross-types in the March, April, June, and August samples but found none (Fig. 4). For this reason and because the number of full-sib families compared was small (three to six), we concluded that a determination of broad sense heritability of seawater adaptability within cross-types would have little meaning.

The significant cross-type effects apparent in the overall comparison of the hatchery population were also apparent in the more rigorous analysis (B) using only the independent family means (Fig. 4; Table 2). Differences in seawater adaptability apparent in the hatchery populations reflect real differences between sockeye and kokanee in the wild.

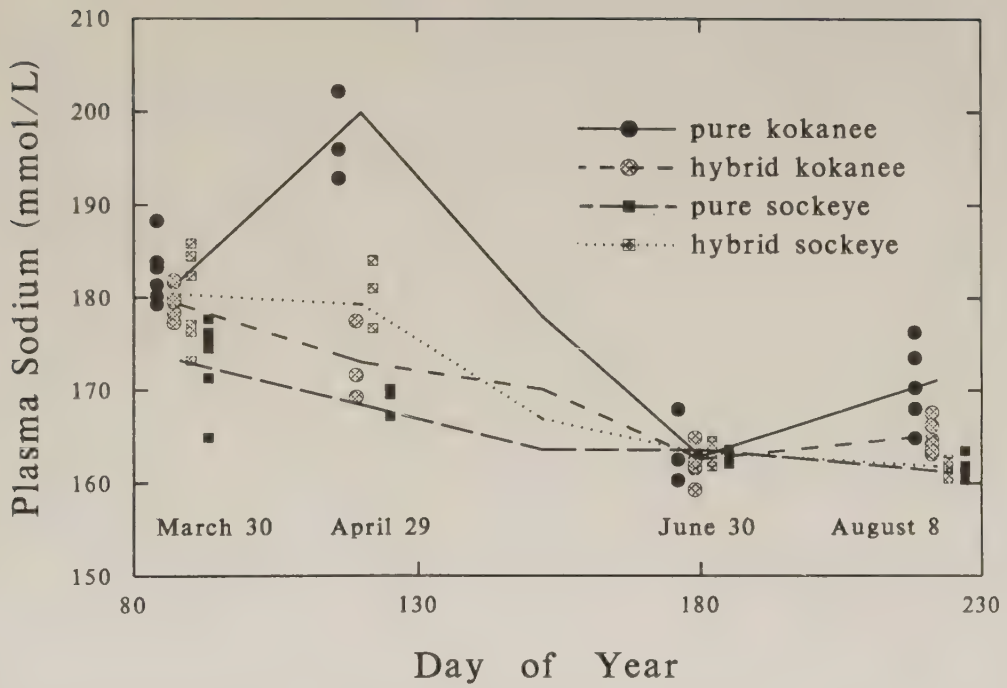

FIG. 4. Mean plasma sodium concentrations of full-sib families of yearling sockeye, kokanee, and their reciprocal hybrids (hybrid type named after the female parent) after 24 h of exposure to natural seawater (29–30‰) during the March, April, June and August sampling periods. The symbols depicting individual family means are staggered by cross-type within each sample period for viewing convenience. Lines denoting each group are derived from all fish sampled. Note that these data required log transformation prior to ANOVA.

Male type effects were most apparent in the April sample. In six of eight maternal half-sib family pairs with replicate samples (five PK and HK family pairs and three PS and HS family pairs), progeny sired by the sockeye males showed, on average, significantly greater adaptability to seawater ($P < 0.05$, t-tests). In the remaining two pairs of maternal (sockeye) half-sib families, those sired by a sockeye male again showed superior adaptability to seawater on average, but the results were not significant. Note that significant differences between half-sib families were observed even though sample sizes were relatively small for all comparisons (5.2 progeny per family, on average).

Discussion

Sockeye and kokanee display an annual cycle in their seawater adaptability in the spring and early summer and the lowest adaptability. In general, both forms showed the highest seawater adaptability in midwinter (independent of rearing density), a pattern typical of anadromous salmonids (Hoar 1976, 1988; Clarke et al. 1985). These results demonstrate that this important aspect of the seasonal smolting cycle of anadromous sockeye has not been lost in nonanadromous kokanee. Nevertheless, striking and consistent differences were observed between the forms in both the timing and duration of the ability to regulate plasma sodium and hence to adapt to seawater.

Sockeye yearlings first showed increased adaptability to seawater by late March and were fully tolerant of seawater by late April (i.e. plasma sodium < 170 mmol/L as typical of smolts; Clarke 1982). Seawater tolerance was maintained through the August sample period. In contrast, kokanee yearlings showed a continual decline in their ability to regulate plasma sodium levels over the winter and early spring, punctuated by near to sometimes lethal levels of plasma sodium in late April. Thereafter, kokanee displayed a rapid increase in seawater adaptability, but still did not display a typical smolt's ability to regulate sodium until late June. Even then, this response to seawater was apparently maintained only until early August.

Reduced seawater tolerance has been observed previously in nonanadromous Atlantic salmon (Barbour and Garside 1983; Burton and Idler 1984; Birt and Green 1986). However, at least some nonanadromous Atlantic salmon populations have maintained seasonal adaptability to seawater, although their ability to tolerate seawater still appears to be less than that of anadromous populations (Evropeizeza 1962; Barbour and Garside 1983; Chernitskiy and Loenko 1983; Birt et al. 1991). The results of this study point to the need to examine aspects of the smolting cycle over time in comparative studies, as the cycles of anadromous and nonanadromous forms may be asynchronous.

The differences in the ontogeny of seawater adaptability between sockeye and kokanee are almost undoubtedly genetic in origin. This argument is supported by the consistent significant male and female type effects observed during the natural sockeye smolting period (March, April, May 1988). Moreover, striking differences were observed between maternal half-sibs sired by sockeye and kokanee males in April. Maternal half-sibs sired by sockeye males showed consistently higher ability to regulate plasma sodium than their half-sibs sired by kokanee. Finally, yearling reciprocal hybrid groups were generally intermediate in their seawater adaptability, as would be expected from additive genetic inheritance of this component of the parr–smolt transformation.

Nongenetic explanations for the observed differences between sockeye and kokanee, such as tank effects, growth effects, and differences in maturation, can be largely ruled out. Rearing location had only a slight quantitative but no qualitative effect on seawater performance of the cross-types. Fish reared in pooled groups at the Pacific Biological Station responded almost identically to those raised in separate tanks at the Rosewall Creek Hatchery when challenged in seawater.

Larger fish are typically more tolerant of seawater than smaller fish, other things being equal (e.g. Conte et al. 1966; Leatherland and McKeown 1974; Hoar 1976; McCormick and Saunders 1987). Negative correlations between fork length and plasma sodium concentrations within groups were common in this study. However, size differences among groups were negligible (see Wood and Foote 1990) and we corrected for any small differences that did exist. It is readily apparent that seawater adaptability in *O. nerka* is more than just an effect of size; the ability to regulate plasma sodium cycled over the seasons even though all cross-types increased relatively uniformly in size (Tables 1 and 2).

Precocious maturation in males is associated with reduced seawater tolerance in salmonids (Saunders et al. 1982; Aida et al. 1984; Langdon and Thorpe 1985; Thorpe 1987; Dellefors and Faremo 1988; Foote et al. 1991). Thorpe (1986, 1987) has argued that the processes of smolting and maturation are mutually incompatible. At least part of this incompatibility appears to be associated with the release of androgens during maturation (Lundqvist et al. 1989). Given that the extent of yearling (precocious) maturation was much higher in kokanee than in sockeye, we suspected that the differences observed between forms may have simply reflected differences in the state of maturation and not differences in seawater adaptability, per se. However, this was not the case. Unlike studies on other species, we found no indication that the process of maturation had an inhibitory effect on the ability to regulate plasma sodium until the very final stage of maturation. In short, differences among cross-types were not the result of differential maturity; male and female type effects on seawater adaptability were equally evident in analyses using only nonmaturing individuals.

The reason(s) why the process of maturation did not inhibit hypoosmoregulatory ability in kokanee and the reciprocal hybrids until the final stages of maturation remain unknown. However, it is interesting to note that maturing males did not differ from nonmaturing males in their growth characteristics and colour as is typical in other salmonids (e.g. Rowe and Thorpe 1990; Foote et al. 1991). Whereas precocious males in other salmonid species typically maintain distinct parr marks and develop relatively deep bodies in comparison with silver and slender smolts, precocious males in this study remained silver and showed no change in relative growth patterns until the final stages of maturation. In this sense, freshwater maturation in *O. nerka* appears more similar to the marine maturation of anadromous salmonids where the fish remain silver and slender until, and inhibitory effects on seawater tolerance are limited to, the final stages of maturation (Boykov et al. 1988) than it is to the precocious maturation of nonmigrant parr in other salmonids.

The development of high seawater adaptability exhibited by sockeye by late April is undoubtedly adaptive, as it coincides nearly exactly with the natural timing of the smolt migration from Shuswap Lake (see Birtwell et al. 1987). Fish that are not able to adapt to seawater by the time they reach the sea will be physiologically stressed, resulting in either death or stunted growth (Hoar 1976; Clarke 1982; Saunders et al. 1985; Clarke and Shelbourn 1986; McCormick and Saunders 1987; Järvi 1990).

In contrast, it is not clear whether the seasonal cycle in seawater adaptability is beneficial for kokanee. The overall smolting process appears energetically costly in salmonids (Hoar 1976), with consequent negative effects on the immune system (Maule et al. 1987) and ability to handle stress (Järvi 1990). Given this potential cost, it is possible that the maintenance of at least some of the components of the smolting cycle in kokanee simply reflects the close relationship and perhaps interbreeding between sympatric sockeye and kokanee (Foote et al. 1989). However, this does not appear to be the case. Kokanee that have been isolated from the sea (and hence from sockeye) in Kootenay Lake for over 10 000 yr still show a seasonal cycle in their ability to regulate plasma sodium when placed in seawater, demonstrating that the response is not maintained by recent gene flow from sockeye (C. Foote, C. Wood, C. Clarke, and J. Blackburn, unpubl. data; see also Leatherland and McKeown 1974; Schmitz 1989). However, we cannot rule out the possibility that there has been insufficient time for kokanee populations in Shuswap and Kootenay lakes to lose this component of the smolting response given that these areas were previously glaciated (McPhail and Lindsey 1986) and that kokanee appear to have arisen from colonizing sockeye since deglaciation (Ricker 1940; Nelson 1968; Foote et al. 1989).

It is conceivable that at least some components of smoltification may be advantageous to kokanee in their lacustrine life (Hoar 1976). For example, the general silvering of body pigmentation is associated with smoltification (Johnston and Eales 1967; Eriksson and Lundqvist 1982) but may also be advantageous for *O. nerka* in a lacustrine environment given that the silver colouration of smolts is undoubtedly cryptic in both the marine and lacustrine pelagic environments. In addition, smolting is associated with an increase in output of growth hormone (Hoar 1988), which may be advantageous to both a marine and freshwater environment, given its association with both increased growth (Higgs et al. 1976) and seawater adaptability (Clarke et al. 1977). The plausibility of this suggestion is supported by Leatherland et al.'s (1974) discovery of greater activity of growth hormone cells in kokanee "smolts," indicating that this trait typical of smolts has not been lost in the nonanadromous form of the species. Similarly, smolting is usually associated with a change from aggressive territorial behaviour in fresh water to schooling behaviour during migration and in the sea. Given that *O. nerka* appears to be a nonaggressive schooling species in both the freshwater and marine environments (Hoar 1976), there is the possibility that this nonaggressive behaviour is maintained by a release or suppression of hormones associated with the seasonal smolting response. Finally, increased thyroid output which is commonly associated with smolting (see Dickhoff and Sullivan 1987) may be adaptive in the uptake of iodine, which is scarce in the freshwater environment (Hoar 1976). Therefore, it is possible that the seasonal cycle in seawater adaptability is maintained in kokanee as a correlated trait of selection on these associated factors.

It is probable that hybrids of sockeye and kokanee would experience reduced fitness if they migrated to sea. The survival value of migratory timing has been well documented in hatchery release experiments (Bilton et al. 1982). The onset of increased seawater adaptability was delayed in the reciprocal hybrid groups compared with sockeye. Hybrids migrating to sea later than sockeye would probably be exposed to increased

predation without the swamping effect of the mass migrations of sockeye smolts (e.g. Ward and Larkin 1984; Wood 1987). On the other hand, hybrids migrating at the same time as sockeye would be more prone to osmotic shock because of their decreased seawater adaptability (Clarke 1982). In this sense, kokanee genes invading a sockeye population may have a deleterious effect on seawater adaptability and swimming performance (Taylor and Foote 1991) similar to that of some parasites (Boyce and Clarke 1983).

In contrast, hybrids remaining in fresh water may not suffer any deleterious osmotic effects, given that kokanee appear relatively unimpeded by their seasonal cycle in seawater adaptability. At this point, we do not know whether natural hybrids survive to smolt, and if they do, whether they migrate to sea or remain in the lake.

In conclusion, we have shown that sympatric sockeye and kokanee differ genetically in the timing of their seasonal adaptation to seawater. However, the difference between forms is quantitative and not qualitative. Even kokanee were able to grow to maturity in seawater, when transferred in late June 1988 once they had developed high seawater adaptability (C. Wood and C. Foote, unpubl. data). Hence, it seems unlikely that these differences alone can account for the observed reproductive isolation between forms. We have previously documented genetic differences between sympatric sockeye and kokanee in development, growth, and swimming performance which are presumably adaptive (Wood and Foote 1990; Taylor and Foote 1991). In addition, we have found no evidence of decreased fertility in hybrid versus pure forms (C. Wood and C. Foote, unpubl. data). Consequently, it appears that selection against hybrids of the two forms acts throughout their divergent life histories, rather than at any single, lethal stage. Presumably, such selection has led to the evolution of the prezygotic isolating mechanisms, in addition to size, that exist between the forms (Foote 1987; Foote and Larkin 1988).

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Systemic and Enzymatic Responses to Endurance Training in Two Cyprinid Species with Different Life Styles (Teleostei: Cyprinidae)

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Hinterleitner, S., M. Huber, R. Lackner, and W. Wieser. 1992. Systemic and enzymatic responses to endurance training in two cyprinid species with different life styles (Teleostei: Cyprinidae). Can. J. Fish. Aquat. Sci. 49: 110–115.

Juveniles of the zooplanktivorous Danube bleak (*Chalcalburnus chalcoides mento*) and of the benthivorous nase (*Chondrostoma nasus*) were subjected to an endurance training regime for 81–90 d. Growth performance was better in trained than in untrained Danube bleak but not in nase. Mass specific heart size was significantly greater in trained than in untrained *C. nasus*, but no difference was found in *C. chalcoides*. The size of muscle fibres increased with training in both species; in nase the red and pink fibres and in Danube bleak the red and white fibres exhibited the most conspicuous response. Mass specific activities of the enzymes of energy metabolism were generally not affected by training, but in the white fibres of *C. chalcoides*, three glycolytic enzymes displayed a parallel trend indicating an increase of the glycolytic potential with training of about 25%. Since the Danube bleak maintains an irregular, burst-like mode of swimming even under the endurance training regime, we assume the lactate produced intermittently in the white muscles to be oxidized in the heart and the red muscles. The activities of three glycolytic heart enzymes were up to 25-fold higher in *C. chalcoides* than in *C. nasus*.

Des juvéniles de *Chalcalburnus chalcoides mento* zooplanctivores et de *Chondrostoma nasus* benthivores ont fait l'objet d'un régime d'exercice d'endurance pendant une durée de 81 à 90 jours. La croissance a été supérieure chez les *C. chalcoides* ayant subi le régime d'exercice mais non chez les *C. nasus*. La taille du coeur, déterminée par la masse spécifique, a été plus élevée de façon significative chez les *C. nasus* ayant subi le régime, mais aucune différence n'a été notée chez l'autre espèce. Chez les deux espèces, la taille des fibres musculaires a augmenté avec l'exercice. Les réponses les plus apparentes ont été notées pour les fibres rouges et roses, chez *C. nasus*, et pour les fibres rouges et blanches, chez *C. chalcoides*. Les activités spécifiques à la masse des enzymes du métabolisme énergétique n'ont généralement pas été affectées par l'exercice, mais on a noté, pour les fibres blanches de *C. chalcoides*, que trois enzymes glycolitiques présentaient une tendance parallèle démontrant une augmentation d'environ 25 % du potentiel glycolytique avec l'exercice. Comme les *C. chalcoides* ont maintenu une nage irrégulière et saccadée même en régime d'exercice, nous avons supposé que le lactate produit de façon intermittente dans les muscles blancs fait oxydé dans le coeur et les muscles rouges. Les niveaux d'activité de trois enzymes glycolytiques du coeur étaient jusqu'à 25 fois plus élevées chez *C. chalcoides* que chez *C. nasus*.

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Numerous investigations have been conducted on the effects of training in fish, but so far no general picture, comparable with that for mammals, has emerged (for review see Davison 1989). This is undoubtedly due to differences in the methods used (particularly with regard to swimming speed and feeding) and to species-specific features of the responses of fish to training. Moreover, while mammals used for training were fully grown, the fish subjected to training regimes have always been juveniles, with a considerable growth potential. The extent to which this potential was realized may have influenced the results of the experiments.

The main purpose of the present investigation was to compare the effects of endurance exercise training on two closely related species differing in life style, particularly in their swimming mode and feeding habits. The Danube bleak (*Chalcalburnus chalcoides mento*) is a fast-swimming zooplanktivorous and surface-feeding species, the swimming muscles of which are characterized by an exceptionally large glycolytic potential (Hinterleitner et al. 1989; W. Wieser and K. Cerny, unpublished). The nase (*Chondrostoma nasus*) is a typical bottom feeder, grazing on plants, particularly green algae and diatoms growing on stones (Lelek 1987). We hypothesized that differences between the life styles and swimming performances of the two species would be reflected in their responses to an endurance training regime.

Material and Methods

Fish

One-year-old fish were used in this investigation. Each species was represented by a homogeneous group, derived from single parents, initial weights approximating, on average, 2 g (*C. nasus*) and 5 g (*C. chalcoides*) wet body weight. The fish had been bred and maintained in running-water aquaria at 18–20°C and fed dry food, *Tubifex*, and *Artemia* nauplii ad libitum. The experiments with *C. nasus* were conducted in 1988–89 and those with *C. chalcoides* in 1989–90.

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Training

The training flume, used in a previous investigation (Lackner et al. 1988), was constructed of PVC plates and was subdivided into four compartments so as to allow the simultaneous treatment of up to four groups of fish. Two pumps (746 and 1119 W) were used to recirculate the water and to maintain the flow. A filter consisting of Perlon wool was installed in a bypass of the circulation system. Grids of stainless steel and PVC were used to rectify and to regulate the water current. Trained fish were forced to swim against a current of 25 $cm \cdot s^{-1}$ which was equivalent to 2.5–3.5 body lengths$\cdot s^{-1}$. The control fish were kept at 3 $cm \cdot s^{-1}$. The temperature in all experiments was 20°C. Fish were fed four times a day with commercial carp food (Alma, FRG) by means of an automatic feeder, 4–5% of the fish mass being the daily ration. During each feeding period of 20 min the flow of the flume was automatically reduced to the control level. A daily regime of 15 h light and 9 h dark was maintained throughout the experiments, but the flow regime was not altered during the night. Groups of fish were removed from the flume for further study after 90 d (*C. nasus*) or 81 d (*C. chalcoides*) of training.

Preparation of Fish and Tissues

At the end of the experiment, wet body weight (BW, grams), total length (TL, centimetres), and condition factor ($CF = BW \cdot 100 \cdot TL^{-3}$) were determined for each fish. Hearts were excised, washed in saline, lightly blotted between filter paper, and weighed. Relative heart weight (HW) is given as HW (milligrams)/BW (grams). For the morphometry of swimming muscles, fish were decapitated and descaled. Thick slices (about 0.5 cm) of lateral muscles were excised, embedded in Tissue Tek II O.C.T. compound, and frozen in liquid propane cooled with liquid nitrogen. Muscle slices of both species derived from the anal region (immediately posterior to the anus) will be compared.

Histology and Histochemistry

Prior to sectioning, the samples that had been cooled in liquid nitrogen were allowed to equilibrate to the cutting temperature of −15 to −20°C in a refrigerated cryostat cabin for 1 h. Serial transverse sections were cut at 10 μm and mounted on glass coverslips. Histochemical tests for myofibrillar ATPase and cytochrome oxidase were used to differentiate between red, pink, and white fibres of the swimming muscles. For the demonstration of myofibrillar ATPase, sections were stained by a modification of the method of Guth and Samaha (1970): alkaline preincubation (pH = 10.4) was used to differentiate between fibre types. In controls, ATP was omitted from the incubation medium and sodium azide was added to inhibit mitochondrial ATPase activity. Sections were stained for cytochrome oxidase (COX) using the method of Lojda et al. (1976). Controls were incubated for the same length of time in a medium in which cytochrome *c* was replaced by an equal volume of buffer.

For the quantification of histological parameters, representative sections were photographed through a Polyvar (Reichert-Jung) microscope. The diameters of red, pink, and white muscle fibres were calculated from photographic prints by means of a digitizer tablet (Summagraphics MM 1201). Between 35 and 80 fibres of each type were measured per individual fish. To obtain a random sample, all fibres present in each of a number of frames were measured. Calculation of differences was based on data of at least four specimens from each treatment group. Red muscle fibres were counted on panoramic views of lateral body regions obtained by combining photographic prints of the individual sections. Drawings of whole-body transverse sections were used to calculate the relative contribution of red muscle fibres to total swimming musculature.

Enzymes

Fish were killed by a blow on the head. Liver, heart ventricle, and red and white muscle sampled from the last third of the upper part of the trunk were quickly dissected, blotted dry, and weighed. The tissues were stored in a Biofreezer at −70°C until required. They were homogenized with an Ultraturrax homogenizer in ice-cold extraction medium at constant dilution (0.015 g sample·1 mL $medium^{-1}$). The homogenates used for the determination of PFK, PK, LDH, CK, and MK activity were centrifuged at 25 000 × *g* for 20 min and those for COX and Thiolase at 600 × *g* for 15 min. The crude homogenate was used for the determination of the OGDH activity.

Enzyme activity was measured at 20°C. Results are given in units (1 U = 1 $\mu mol \cdot min^{-1}$) per gram fresh weight. Optimal extraction and assay conditions for individual enzymes are given below (assay conditions refer to final concentration of components in the measuring cuvette).

Phosphofructokinase (PFK, EC 2.7.1.11). Extraction medium: 50 mM Tris, pH 8.2, 1 mM EDTA, 5 mM $MgCl_2$. Assay medium: 50 mM Tris, pH 8.2, 6 mM $MgCl_2$, 200 mM KCl, 1 mM dithioerythritol (DTT). PFK was measured as described by Opie and Newsholme (1967) using 2 mM AMP, 1 mM ATP, 0.17 mM NADH, 3 mM fructose-6-phosphate (F6P), 100 μL CN reagent (0.272 g KH_2PO_4, 0.006 g EDTA, 0.1 g albumin, 0.078 g KCN in 100 mL a.d.), excess coupling enzymes aldolase, α-glycerophosphate dehydrogenase, and triosephosphate isomerase.

Lactate dehydrogenase (LDH, EC 1.1.1.27). This was assayed according to Bergmeyer (1983). Extraction medium: 0.067 M phosphate buffer, pH 7.5. Assay medium: 0.067 M phosphate buffer, pH 7.5, with 0.15 mM NADH, 1 mM sodium pyruvate.

Pyruvate kinase (PK, EC 2.7.1.40). Extraction medium: as for pyruvate reductase. Assay medium: 0.067 M phosphate buffer, pH 7.5, 70 mM KCl, 4 mM $MgCl_2$, 2 mM ADP, 0.15 mM NADH, 2 mM phosphoenolpyruvate (PEP), 4 U LDH$\cdot mL^{-1}$.

Creatine kinase (CK, EC 2.7.3.2). Extraction medium: 50 mM Pipes/NaOH buffer, pH 7.0, 1 mM EDTA, 4 mM DTE, 3 mM magnesium acetate, 30 mM KCl, 0.1% Triton X-100. Assay medium: extraction buffer except Triton X-100, 0.5 mM $NADP^+$, 0.2 mM ADP, 9.1 mM AMP, 14.5 mM glucose, 18 mM creatine phosphate, 1 U hexokinase$\cdot mL^{-1}$, 0.33 U glucose-6-phosphate dehydrogenase$\cdot mL^{-1}$.

Myokinase (MK, EC 2.7.4.3). Extraction medium: as for CK. Assay medium: 80 mM K_2HPO_4, 50 mM KCl, 0.75 mM $MgCl_2$, pH 7.5, 0.2 mM NADH, 0.39 mM PEP, 1.2 mM ATP, 1.4 mM AMP, 7 U PK$\cdot mL^{-1}$, 20 U LDH$\cdot mL^{-1}$.

Cytochrome oxidase (COX, EC 1.9.3.1). Extraction medium: 0.1 M phosphate buffer, pH 7.0, 0.1% Triton X-100. Assay medium: 0.01 M phosphate buffer pH 7.0, 1% reduced cytochrome *c*. COX activity was assayed according to Boström and Johansson (1972) by following the oxidation of reduced cytochrome *c* at 550 nm. $\epsilon_{redox} = 19.1$.

Acetoacetyl-CoA thiolase (Thiolase, EC 2.3.1.9). Extraction medium: 75 mM Tris–HCl, pH 7.5, 3 mM $MgCl_2$, 5 mM EDTA, 1 mM DTE, 0.1% Triton X-100. Assay medium: 50 mM Tris–HCl, pH 7.5, 50 mM $MgCl_2$, 0.07 mM acetoacetyl-CoA, 0.1 mM CoA. Reactions were started by the addition of CoA and monitored at 303 nm. $\epsilon = 20.5$.

Oxoglutarate dehydrogenase complex (OGDH). Extraction medium: 50 mM triethanolamine–HCl buffer, pH 7.4, 1 mM EDTA, 1 mM DTE, 2 mM $MgCl_2$, 2 M glycerol, 0.1 mM oxoglutarate. Assay medium: 50 mM triethanolamine–HCl buffer, pH 7.4, 1 mM EDTA, 1 mM DTE, 2 mM $MgCl_2$, 2 M glycerol, 0.1% Triton X-100, 100 μL CN reagent (as for PFK), 2 mM NAD^+, 0.5 mM CoA, 2 mM oxoglutarate.

All chemicals were obtained from Merck, Darmstadt, BRD, and from the Sigma Chemical Company, St. Louis, MO.

Statistics

Significance of differences between means was tested with a two-tailed Student *t*-test. In case of significant differences between variances, a Welch correction was applied.

Results

Whole Body and Heart Size

Untrained and trained *C. nasus* did not differ from each other in body mass or condition factor after 90 d of treatment whereas trained *C. chalcoides* weighed 35% more than untrained ones and had a significantly higher condition factor after only 81 d of exercise (Table 1). An opposite trend was displayed by relative heart size which was significantly greater in trained than in untrained nase but not in Danube bleak. This indicates that in the latter species, heart and body mass increased isometrically with training whereas in trained nase the presumed improvement of circulatory performance (suggested by the increase in relative heart size) was not accompanied by an improvement of growth performance.

Muscle Morphometrics

Endurance training led to an increase of the proportion of muscle composed of red fibres in *C. nasus* but not in *C. chalcoides*. In trained, relative to untrained, *C. nasus* the average diameter of red fibres increased by about 13% whereas the white fibres remained unaffected (Table 2). In contrast, the red and white fibres of *C. chalcoides* increased isometrically, each by about 15%, which explains why in this species the relative contribution of red muscle to total muscle mass remained invariant in trained and untrained specimens. Since about 90% of total muscle mass consists of white fibres, their size increase in *C. chalcoides*, but not in *C. nasus*, accounts for the species-specific growth responses to training observed (Table 1). However, the most strikingly specific response was displayed by the pink muscle fibres, the size of which remained unaffected by training in the Danube bleak but increased by as much as 40% in the nase. In Table 2 the differences in fibre size between trained and untrained groups are based on the mean values of individual fish. A different approach is provided by comparing the distribution of size classes of muscle fibres in the two groups of fish. For this purpose the data from all specimens within each group were pooled and the abundance of arbitrarily defined size classes determined. Pooling was justified because within each group of fish the distribution of fibre size did not differ significantly between individuals.

The following conclusions could be drawn: both red and white muscle fibres were, on average, larger and their size distributions more heterogeneous in *C. chalcoides* than in *C. nasus* (Fig. 1 and 2), and the size distribution of red fibres was strongly affected by training in both species whereas this was true for the white fibres of *C. chalcoides* only (Table 2).

Enzyme Activity

None of the enzymes studied showed a strong response of weight-specific activity to the training regime. Only three differences between untrained and trained fish were significant at the $p = 0.05$ level, two of them signalling a decrease in specific activity with training (Tables 3 and 4). All other enzymes were either totally unaffected by training or displayed nonsignificant trends. Most of these trends suggested a downward shift with training, but the specific activity of each of the three glycolytic enzymes, PFK, PK, and LDH, in the white muscle fibres of *C. chalcoides* increased by about 25% which despite the lack of statistical validation of the activity change of individual enzymes suggests a general enhancement of the glycolytic potential in this species. This suggestion is further supported by the high activities of glycolytic enzymes in the heart of *C. chalcoides* which surpass those of *C. nasus* up to 25-fold.

Discussion

In some respects our data support the general idea that, compared with mammals, fish are less flexible in their responses to endurance training (Davison 1989). This refers particularly to the specific activities of the enzymes of aerobic metabolism in the swimming muscles which, as in brook trout (*Salvelinus fontinalis*) and coalfish (*Pollachius virens*) (Johnston and Moon 1980a, 1980b), showed only slight changes with training. In contrast, endurance training may accelerate growth (Davison and Goldspink 1977; Nahhas et al. 1982; Davie et al. 1986;

TABLE 1. Body mass (g), condition, and heart factor (mg heart/g body) in untrained (U) and trained (T) specimens of two cyprinid species. Duration of training period was 90 d in *C. nasus* and 81 d in *C. chalcoides*. Mean values, SD, and number of fish (in parentheses) are indicated; *p*-values based on Student's *t*-test (NS = not significant).

	C. nasus			*C. chalcoides*		
Status	Mass	Condition	Heart	Mass	Condition	Heart
U	4.64 ± 1.05 (63)	0.57 ± 0.03 (57)	0.57 ± 0.08 (61)	6.77 ± 1.53 (42)	0.60 ± 0.05 (41)	0.71 ± 0.1 (42)
T	4.84 ± 1.22 (54)	0.59 ± 0.04 (54)	0.66 ± 0.09 (61)	9.12 ± 2.04 (36)	0.64 ± 0.04 (36)	0.73 ± 0.1 (35)
p	NS	NS	<0.001	<0.001	<0.01	NS

TABLE 2. Properties of red, pink, and white muscle fibres of untrained (U) and trained (T) specimens of two cyprinid species. n = total number of fibres; fibre diameter in μm; % = volume of red fibres in % of total muscle mass. Mean, SD. and number of examined fish (in parentheses) are indicated. Difference between untrained and trained fish is given as probability, based on Student's t-test, and percent change of trained compared with untrained specimens (NS = not significant).

Species	Red fibres			Pink fibre diameter	White fibre diameter
	n	Diameter	%		
C. nasus					
U	1388 ± 67(3)	27.8 ± 1.2(4)	5.7 ± 0.5(8)	32.0 ± 3.0(4)	47.1 ± 2.3(8)
T	1529 ± 95(3)	31.4 ± 1.9(5)	7.5 ± 0.2(6)	45.2 ± 4.0(4)	50.5 ± 3.9(6)
Change					
p	NS	<0.01	<0.001	<0.01	NS
%	—	12.9	31.6	41.2	—
C. chalcoides					
U	1299 ± 226(3)	33.8 ± 2.3(4)	9.2 ± 0.7(7)	51.2 ± 3.1(4)	59.7 ± 2.7(4)
T	1560 ± 118(2)	38.7 ± 3.0(4)	9.3 ± 0.9(10)	54.7 ± 4.1(4)	68.7 ± 4.8(4)
Change					
p	NS	<0.05	NS	NS	<0.05
%	—	14.5	—	—	15.1

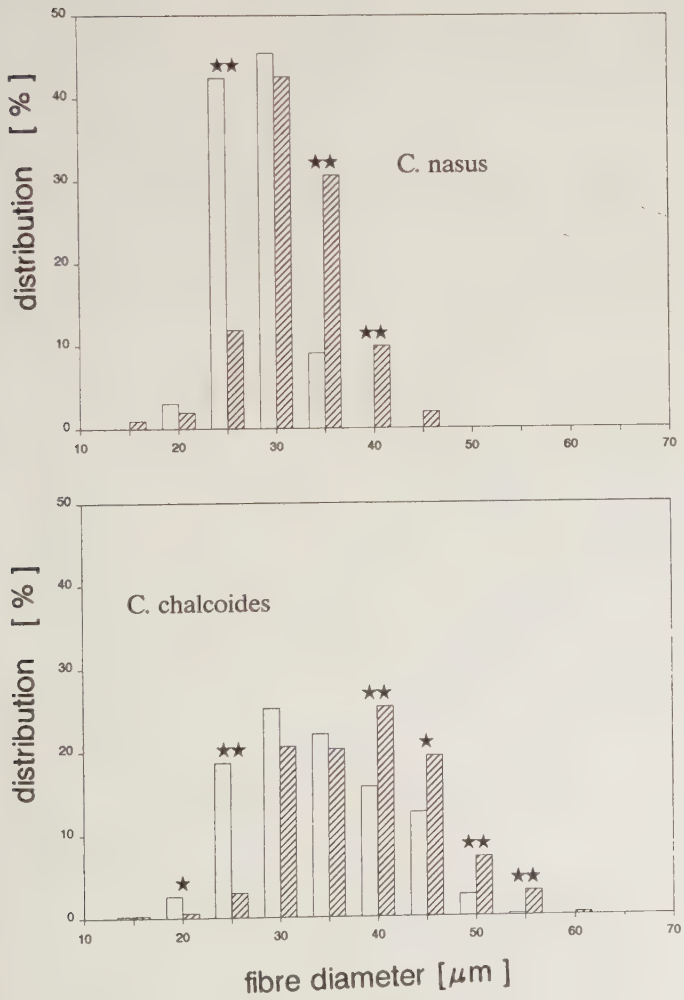

FIG. 1. Frequency distribution histograms of size classes of red muscle fibre diameter for trained (hatched bars) and untrained (open bars) *C. nasus* and *C. chalcoides*. Difference between groups significant at $*p < 0.05$ or $**p < 0.01$.

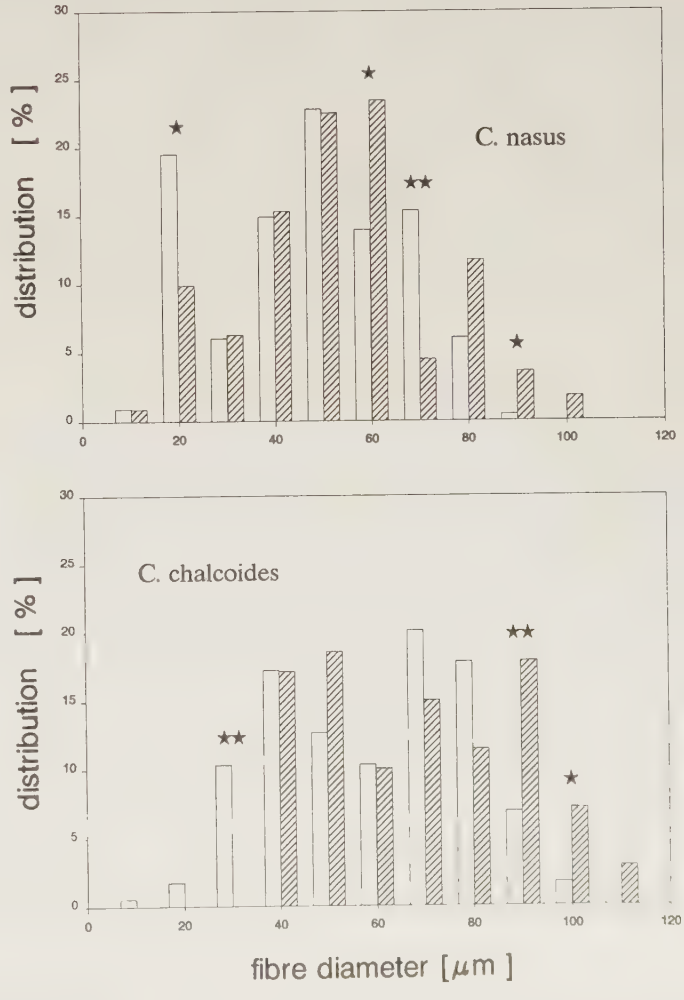

FIG. 2. Frequency distribution histograms of size classes of white muscle fibre diameter for trained (hatched bars) and untrained (open bars) *C. nasus* and *C. chalcoides*. Difference between groups significant at $*p < 0.05$ or $**p < 0.01$.

TABLE 3. Enzyme activities (μmol·min $^{-1}$·g fresh weight $^{-1}$) in three tissues of untrained (U) and trained (T) juvenile *C. nasus*. Training period was 90 d; $n = 5$ fish; difference significant at $^{*}p \leq 0.05$.

	Heart		Red muscle		White muscle	
Enzyme	U	T	U	T	U	T
PFK	0.4 ± 0.05	0.34 ± 0.1	4.0 ± 1.0	3.9 ± 0.9	4.0 ± 0.8	4.2 ± 1.2
PK	60.2 ± 3.5	59.4 ± 13	80.4 ± 11	58.0 ± 26	62.0 ± 23	51.9 ± 3.3
LDH	164 ± 16	145 ± 20	193 ± 47	164 ± 44	152 ± 53	154 ± 33
Thiolase	4.2 ± 0.4	4.4 ± 0.5	5.3 ± 0.5	4.5 ± 1.8	0.2 ± 0.05	0.24 ± 0.1
OGDH	2.7 ± 0.7	3.1 ± 0.9	4.8 ± 0.7	5.7 ± 0.8	0.17 ± 0.03	0.24 ± 0.05*
COX	68.2 ± 7.3	66.1 ± 4.9	40.2 ± 8.2	36.5 ± 3.1	3.3 ± 0.5	3.4 ± 0.8
MK	52.3 ± 19.4	50.8 ± 26	200 ± 57.4	166 ± 30	533 ± 50	394 ± 57*
CK	87.3 ± 13.5	90 ± 34.5	507 ± 79	434 ± 53	829 ± 130	974 ± 312

TABLE 4. As for Table 3, but for *C. chalcoides* after a training period of 81 d.

	Heart		Red muscle		White muscle	
Enzyme	U	T	U	T	U	T
PFK	9.7 ± 4.2	9.0 ± 2.7	8.9 ± 1.3	6.9 ± 1.2	7.8 ± 2.6	9.9 ± 4.2
PK	173 ± 8.4	120 ± 14*	89.2 ± 10	77.2 ± 6.3	107 ± 18.4	131 ± 11.3
LDH	505 ± 54.3	350 ± 50	173 ± 21.2	187 ± 14	303 ± 14	376 ± 54.7
Thiolase	7.2 ± 0.3	7.0 ± 2.4	10.3 ± 1.2	8.9 ± 0.9	0.6 ± 0.2	0.4 ± 0.2
OGDH	4.1 ± 1.7	3.5 ± 1.8	3.3 ± 1.0	2.6 ± 1.1	0.14 ± 0.06	0.15 ± 0.04
COX	98 ± 23	68.3 ± 17	45.6 ± 19.4	32.0 ± 2.2	3.1 ± 0.9	2.04 ± 0.9
MK	108 ± 23	113 ± 1.8	315 ± 26.5	278 ± 29	462 ± 4.8	481 ± 15
CK	168 ± 8.3	204 ± 50.5	892 ± 40	829 ± 97	1095 ± 34	1100 ± 22

Leon 1986) and protein turnover (Houlihan and Laurent 1987), increase heart size (Hochachka 1961; Greer-Walker and Emerson 1978), and lead to both hyperplasia and hypertrophy of muscle fibres (Greer-Walker and Pull 1973; Davison and Goldspink 1977, 1978; Broughton et al. 1980; Johnston and Moon 1980a, 1980b). Thus the general principle may be expressed that the responses of fish to endurance training are systemic rather than cellular whereas in mammals, it is not only the systemic machinery (mainly the circulatory system), but also mitochondria and oxidative enzymes that respond rather strikingly to an endurance training regime (Holloszy and Booth 1976; Hoppeler 1986; Kirkwood et al. 1987). However, our data on two related fish species with different swimming modes and feeding habits permit a more differentiated view of this general principle. In the nase, the variables commonly used as indicators of aerobic capacity, i.e. heart size, relative proportion of red muscle mass, and the activity of OGDH, were enhanced by the training regime. This is in agreement with the responses of other fish species to endurance training, such as roach (*Rutilus rutilus*) (Broughton et al. 1980), brook trout (Johnston and Moon 1980a), and brown trout (*Salmo trutta*) (Davison and Goldspink 1977). In addition, a very pronounced increase in size of the pink fibres was observed in *C. nasus*. The response of the Danube bleak to the training regime was quite different, the most prominent distinguishing feature being the increase of the mass of the white muscle fibres and the constancy of the pink fibre mass. Moreover, total activity of the three key glycolytic enzymes in the white muscles of the posterior body region did not change at all in the nase, but showed a marked parallel trend towards an increased glycolytic potential in the Danube bleak. This is the more remarkable in that even in untrained Danube bleak the glycolytic potential of the white fibres was about twice as high as that of untrained nase (Tables 3 and 4).

One way of explaining the response of the Danube bleak is that in this species, endurance training stimulated preferentially the aerobic glycolytic capacity of the white muscles. This would agree with the observation by Moyes et al. (1989) that in the carp the mitochondria of white muscle appear to be specialized to use pyruvate as a substrate whereas red muscle mitochondria are able to use a wide range of substrates. Furthermore, in a comparative study involving 11 cyprinid species, Sänger (1992) found Danube bleak to possess by far the highest content of mitochondria in the white muscle fibres.

However, the aerobic aspect may not tell the whole story. This is suggested by two observations. The first is the high glycolytic potential even of the untrained Danube bleak, particularly of the heart. The activity of PFK and LDH in this organ are the highest of any cyprinid species studied so far and among the highest ever reported in fish (MacIntyre and Driedzic 1981; Driedzic and Stewart 1982). The second observation is that when swimming in the training flume the nase (as well as all other cyprinid species studied by us: Lackner et al. 1988; and unpublished observations) swim at more or less constant speed whereas the Danube bleak maintains an intermittent, burst-like mode of swimming even under the endurance training regime. Bleak will dart forward until they reach the front end of the flume and then coast backward until they touch the rear end, picking up speed again, and so on. In the wild, this species is a very efficient zooplanktivore, catching prey by means of burst-like attacks. In feeding experiments with five cyprinids the Danube bleak proved to be the species with the fastest attack performance (Winkler and Orellana 1992).

It appears that small Danube bleak raised in the laboratory maintain their burst-like swimming mode even during the endurance training protocol, suggesting that in this species, sustained swimming contains a large component of anaerobic burst activity, the lactate so produced being used primarily by

the heart as a substrate for aerobic energy metabolism. The possibility that training may lead to enhanced transport and catabolism of lactate from white muscle has already been considered by Johnston and Moon (1980a) in their study of the brook trout. In our investigation with juveniles of the related cyprinid *Leuciscus cephalus*, we showed that training leads to a fourfold enhancement of the clearance of lactate from the body if the fish are mechanically stressed (Lackner et al. 1988). The present investigation suggests that in young Danube bleak, lactate may be intermittently produced by the burst-like mode of activity but is cleared rapidly by the increased activity of the heart, perhaps also by the red muscles which have been credited with this ability a long time ago (Wittenberger and Diaciuc 1965). In the hearts of two marine species, ocean pout (*Macrozoarces americanus*) and sea raven (*Hemitripterus americanus*), the catabolism of exogenous lactate is limited by the reaction catalyzed by lactate oxidase (Driedzic et al. 1985). However, LDH activity (measured in the direction of pyruvate reduction) is at least five times higher in the heart of *C. chalcoides* than in the hearts of these two marine species at comparable pyruvate concentrations, and the affinity of the muscle enzyme for lactate is about 10 times greater in *Rutilus rutilus* and other cyprinid species than in the two marine species at their respective environmental temperatures (Wieser et al. 1987, and unpublished observations). Thus the capacity for lactate oxidation may be up to 50 times higher in the hearts of *C. chalcoides* than in those of the two marine species.

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Assessing Trends in Fishery Resources and Lake Water Aluminum from Paleolimnological Analyses of Siliceous Algae

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Lake water aluminum concentrations have a significant influence on the composition of microfossil assemblages of diatoms and chrysophytes deposited in lake sediments. With the paleolimnological approach of multilake datasets in the Adirondack region of New York, USA, we use canonical correspondence analysis to describe past trends in lake water Al. Four lakes, previously investigated regarding acidification and fishery trends, are used to demonstrate that paleolimnological assessment can also provide direction, timing, and magnitude of trends for both toxic metals and fish resources. Additionally, we use weighted averaging regression and calibration to obtain quantitative reconstructions of past lake water Al concentrations. Such reconstructions provide further insight into fishery resource damage and can be compared with modelling results. According to paleolimnological reconstructions, some (albeit few) of the naturally most acidic lakes in the Adirondack region had preindustrial lake water concentrations of inorganic monomeric Al near 4 $\mu mol \cdot L^{-1}$. Although these high concentrations are surprising from a geochemical point of view, they may partially explain the preindustrial absence of fish, as has been independently determined by paleolimnological analysis of phantom midges (*Chaoborus*). Fishery resource deterioration in acidified Adirondack lakes was coincident with major increases in lake water Al concentrations.

La concentration d'aluminium de l'eau des lacs influe de façon appréciable sur la composition des assemblages de microfossiles de diatomées et de chrysophytes se trouvant dans les sédiments lacustres. En appliquant une approche paléolimnologique à des ensembles de données recueillies à partir de plusieurs lacs de la région des Adirondacks de l'État de New-York (É.-U.), nous avons utilisé l'analyse de correspondances canoniques afin de décrire les tendances passées des concentrations d'Al dans l'eau. Quatre lacs, dont les tendances de l'acidification et de la faune aquatique avaient déjà été étudiées, ont servi à démontrer que l'évaluation paléolimnologique pouvait aussi permettre de déterminer l'orientation, le moment d'apparition et l'importance des tendances des métaux toxiques et des ressources halieutiques. De plus, des régressions et des étalonnages de moyennes pondérées nous ont permis de reconstituer les concentrations d'Al qui existaient antérieurement dans les lacs. De telles reconstructions permettent de mieux comprendre les dommages causés aux ressources halieutiques et leurs résultats peuvent être comparés à ceux de travaux de modélisation. Selon les reconstructions paléolimnologiques, certains (bien que peu nombreux) des lacs naturellement les plus acides de la région des Adirondacks présentaient, avant l'ère industrielle, des concentrations d'Al inorganique monomère atteignant presque 4 $\mu mol \cdot L^{-1}$. Des concentrations aussi élevées surprennent du point de vue géochimique, mais elles peuvent expliquer, du moins en partie, l'absence de poisson pendant l'époque pré-industrielle, comme cela peut être montré de façon indépendante par l'analyse paléolimnologique des moucherons du genre *Chaoborus*. La détérioration des ressources halieutiques des lacs acidifiés des Adirondacks a coincidé avec d'importantes augmentations des concentrations d'Al dans leurs eaux.

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Aluminum in lake water is known to poison biota throughout the foodweb (Driscoll 1985; Campbell and Stokes 1985), including fish (Muniz and Leivestad 1980; Baker and Schofield 1980; Hutchinson et al. 1989) and algae (Gensemer and Kilham 1984; Nalewajko and Paul 1985; Pillsbury and Kingston 1990; Smith 1990). Although the mechanisms of toxicity are imperfectly understood for algae, we used population and community responses of siliceous algae to the modern-day Al gradient as a means of reconstructing past concentrations of this metal from algal assemblages preserved in lake sediments. Our paleolimnological approach is crucial to understanding past Al trends because historical chemical data for this metal are not available prior to 1980.

We are interested not only in past Al concentrations but also in past fish status of lakes. In the absence of fish remains in lake sediments and of historical records, we must assess fish

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status indirectly. Our paleolimnological model relates modern algal responses and contemporary fish status to lake water chemistry by means of constrained ordination methods. We have also positioned fossil algal assemblages into this geometric model on the basis of their overall similarities with the modern assemblages. The fossil algal assemblages can thus be related indirectly to modern lake water chemistry and at the same time to present-day fish status. From temporal changes in the algal assemblages we can also infer changes in fish status through time.

We are not implying that Al is the only toxic metal of interest in these lakes, nor that metals are the only chemicals toxic to algae, but Al is the toxic metal for which we have the best data. Much research on the chemistry of Al in surface waters has been conducted in the Adirondack region, in some of the same lakes that have been studied paleolimnologically (Driscoll 1984; Driscoll and Newton 1985; McAvoy et al. 1992). For example, Driscoll et al. (1984) worked in the lakes and streams of the Moose River watershed and showed that, at pH below 5.0, Al contributes significantly to cation concentration, and both total and inorganic monomeric Al concentrations are elevated. Furthermore, monomeric Al binds to lake water organic carbon as a positive linear function of organic carbon concentration. Although much of the Al in Adirondack streams and lakes is complexed with organic matter, significant amounts exist in more toxic "labile" complexes (primarily with water, hydroxide, sulphate, and fluoride). Driscoll and Schafran (1984) described similar speciation of Al at another Adirondack site in this watershed, Dart's Lake, and they explained that the unsatisfactory agglomerative category "dissolved organic carbon" (DOC) further complicates understanding Al-organic complexes. Driscoll et al. (1987) detailed the loss of DOC as water moves through Big Moose Lake in this system, perhaps as the result of complexation with Al. On a seasonal basis, concentrations of both total and monomeric Al were high when acidity was high (pH low), during high flow in winter and spring.

Our objectives in this paper are to (1) explore diatom species composition data in relation to environmental variables and fish presence/absence in softwater lakes of the Adirondack region of New York, (2) investigate the hypothesis that past lake water Al concentrations are strongly correlated with fish loss, and (3) evaluate past fishery resource health and trends accompanying lake acidification in selected study lakes with the insights gained from paleolimnological diatom, chrysophyte, and *Chaoborous* (Diptera: Chaoboridae) remains.

Study Lakes

Acidification Trends

The four stratigraphically analyzed lakes discussed here (Big Moose Lake, Deep Lake, Upper Wallface Pond, Windfall Pond) have been studied in the Eastern Lake Survey (ELS) (Big Moose Lake and Deep Lake; Landers et al. 1988), the Regional Integrated Lake-Watershed Acidification Study (RILWAS) (Big Moose Lake and Windfall Pond; Rudd 1987), and the Paleoecological Investigation of Recent Lake Acidification (PIRLA) project (all four; Charles et al. 1990; Uutala 1990). Charles et al. (1990) described the pH trends in the four lakes, and in this paper we reconstruct monomeric Al concentrations and past fisheries for these four lakes based on siliceous microfossils.

Previous paleolimnological reconstructions for Big Moose Lake indicate pH declines of 1.2 units between the 1950s and 1982, to a low value near 5.0. Past concentrations of total Al were modelled using MINEQL (Charles 1984; Charles et al. 1987), with a modelled increase of 11–15 $\mu mol \cdot L^{-1}$ between 1953 and 1982. Deep Lake was naturally acidic (pH = ~5.4), and it acidified rapidly after 1945 (Charles et al. 1990; Smol and Dixit 1990). Upper Wallface Pond was naturally acidic (pH = ~5.3), and it acidified gradually during this century (Charles et al. 1990; Smol and Dixit 1990). Windfall Pond retains a carbonate–bicarbonate buffering system, and paleolimnological evidence indicates that it has not recently acidified (Charles et al. 1990).

Fish Trends

Uutala (1986, 1990) studied sediment cores from the same four lakes using invertebrate remains (*Chaoborus*) to infer past fishery changes.

Charles (1984) and Schofield and Driscoll (1987) described fisheries declines in Big Moose Lake. Historical fishery data for the Big Moose watershed show that no native species were extirpated by 1932, but by 1948, many species had restricted distributions and the salmonid sport fishery was declining. The number of fish species declined from 14 in 1931 to 7 in 1983, of which four are non-native and introduced since the 1940s (Charles 1984). Brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), and smallmouth bass (*Micropterus dolomieui*) fisheries have failed, and after many futile attempts during 25 yr, stocking programs were halted in 1975. The *Chaoborus* profile from Big Moose Lake indicated that fish were always present in Big Moose Lake (Uutala 1986).

Deep Lake is currently fishless (Kretser et al. 1989; Charles et al. 1990). The *Chaoborus* profile from Deep Lake indicated that fish had disappeared from the lake by a ^{210}Pb date of 1940 (Uutala 1990). Guides reported good brook trout fishing in the 1930s. New York State Department of Environmental Conservation (NYSDEC) records show that the lake was stocked annually with brook trout fingerlings from 1951 to 1968. Only three trout were captured during surveys in 1954, 1964, and 1965, and none were captured during 1966, 1967, and 1968 surveys (references in Smol et al. 1984). These records indicate the poor success of the stocking effort.

Upper Wallface Pond is currently fishless according to NYSDEC records (Charles et al. 1990), and it has no history of a sport fishery. *Chaoborus* stratigraphy indicates that the lake has never had viable fish populations (Uutala 1990). Brook trout fingerlings were regularly stocked from 1958 to 1975, but fish surveys in the early 1960s showed poor growth and survival of the stocked fish. No trout were captured in a 1975 survey (Paterson 1985). The poor survival of the stocked fish was also indicated by the persistance of *Chaoborus americanus* during the period of stocking (Uutala 1990).

The *Chaoborus* profile from Windfall Pond indicates that fish have always been present (Uutala 1990). Fish are currently abundant in Windfall and stocking has not occurred (Colquhoun et al. 1984; D. F. Charles, Indiana University, c/o USEPA Environmental Research Laboratory, Corvallis, OR 97333, USA, pers. comm.; Kretser et al. 1989).

Methods

Diatom and Chrysophyte Data

The Adirondack Park modern diatom – lake chemistry data set has been described previously as part of the PIRLA-I project

TABLE 1. Summary of lake water chemical, physical, and fishery resource data for the 37 lakes used in the diatom ordination and calibration. All chemical values are means for shallow-water, ice-free collections. See text for a description of the data sources for Al and PIRLA-II lake data and Charles et al. (1990) for a description of PIRLA-I lake data.

Lake name	Lake No.[a]	pH	Monomeric Al ($\mu mol \cdot L^{-1}$)[b]	DOC ($\mu mol\ L^{-1}$)	Secchi (m)	Elevation (m)	Fish status[c]
Arbutus Lake	1	6.89	0.1	326	4.7	513	+
Avalanche Lake	2	4.85	8.3	298	4.5	873	−
Barnes Lake	3	4.71	0.6	494	1.8	396	−
Bear Pond	4	4.97	1.9	27	9	499	+
Big Moose Lake	5	5.1	5.3	286	6	556	+
Black Pond	6	7.29	0.3	336	4.6	494	+
Bog Pond	7	5.49	1.8	341	1.8	498	+
Bubb Lake	8	6.53	0.9	265	2.7	554	+
Cat Mt. Pond	9	5.1	1.3	64	7.6	526	+
Clear Pond (E.L.)[d]	10	7.05	0.9	312	6	584	+
Cowhorn Pond	14	6.18	0.2	204	3.6	530	+
Crane Mt. Pond	15	6.58	0.5	176	4.3	793	+
Dart's Lake	16	5.38	4.3	266	6.1	536	+
Deep Lake	17	4.69	10.7	113	8.3	789	−
Deer Lake	18	7.17	0	251	2.9	509	+
Dunk Pond	19	7.55	0.6	392	3.9	474	+
E. Copperas Pond	20	4.48	1.7	371	2.2	480	+
Frank Pond	21	6.62	0.1	153	7.7	517	+
Green Pond	23	6.19	0	239	6.2	493	+
Gull Pond	24	7.26	0	263	4.1	319	+
Heart Lake	25	6.56	0.6	252	4.7	661	+
Huntley Pond	26	7.76	0.1	292	3.5	482	+
Lake Arnold	28	4.83	7.8	266	2.7	1150	−
Lake Colden	29	4.93	8.4	276	6.3	843	−
Lake Rondaxe	30	6.6	1	257	4.9	536	+
Lake Tear of the Clouds	31	4.66	9.3	263	0.8	1320	−
Little Echo Pond	32	4.32	0.6	1058	0.6	482	−
Little Shallow Pond	34	7.29	3.4	154	1.3	515	+
Livingston Pond	35	5.76	0.8	171	6.8	850	+
Moss Lake	38	6.82	0.3	288	5.4	525	+
Mountain Pond	39	4.69	0.2	67	4.4	482	−
Pine Pond	42	6.99	0	136	7.1	476	+
Round Pond	45	6.98	0	186	5.7	527	+
U. Wallface Pond	47	4.96	5.3	110	5.9	948	−
Washbowl Pond	48	4.51	2.6	282	1.2	515	−
Wolf Lake (H.F.)[d]	51	7	0	293	7.6	556	+
Woodruff Pond	52	7.7	0.4	756	1.7	474	+

[a]Corresponds to lake No. in the ordination diagram (Fig. 1) and location map in Charles et al. (1990).

[b]Labile monomeric Al.

[c]+ and − denote fish presence and absence, respectively.

[d]Letters in parentheses allow separation of lakes with similar names within the PIRLA database (notation follows Charles et al. 1990).

(Charles 1985; Charles et al. 1987, 1990). For the purposes of this paper, we used the 37 PIRLA-I lakes for which both fish data and monomeric Al measurements were available in our diatom ordinations, regressions, and calibrations (Table 1). A larger 62-lake dataset that includes both PIRLA-I and -II drainage lakes (Charles and Smol 1990) was used to develop the supporting chrysophyte monomeric Al inferences (Cumming et al. 1992a, 1992b).

Our analyses are based on data for all diatom and chrysophyte taxa present in $\geq 1\%$ relative abundance in at least one and three lakes, respectively. This amounts to 140 diatom and 23 chrysophyte taxa. Laboratory methods follow Kingston (1986) for diatoms and Smol (1986) for chrysophytes. The PIRLA diatom taxonomy is documented in Camburn et al. (1984–86), Charles (1986), and Camburn and Kingston (1986). Chrysophyte taxonomy follows Wee (1982), Takahashi (1978), Smol et al. (1984), and Asmund and Kristiansen (1986). At least 500 diatom valves and 500 chrysophyte scales were counted along transects under oil immersion (1000–1250×, NA = 1.3–1.4). Quality assurance design and implementation are documented in Charles and Whitehead (1986), Kreis (1989), Cumming et al. (1990), and Kingston et al. (1992).

Water Chemistry

A limnological data summary for the PIRLA-I project has been published (Charles 1985; Charles et al. 1990), and detailed data are available from the authors. PIRLA-I chemistry meth-

ods are documented in Cook (1986), and quality assurance is documented in Cook and Kahl (1986). Other sources of water chemistry and watershed data in the PIRLA database (Charles et al. 1989) include ELS-I (Kanciruk et al. 1986), RILWAS (Driscoll and Newton 1985; C. T. Driscoll, Department of Civil Engineering, Syracuse University, Syracuse, NY 13210, USA, pers. comm.), unpublished autumn data from ELS-II (USEPA Corvallis Environmental Research Laboratory), and a 1988 survey of Al chemistry (C. T. Driscoll and A. J. Uutala, pers. comm.).

The pH variable discussed here is the arithmetic mean of ice-free, near-surface samples (collected at either 0.5 m depth in RILWAS and Charles (1985) or 1.5 m in ELS-I and -II, over the deepest part of the lake), equilibrated to atmospheric CO_2 concentration; likewise, the Al values are shallow-water, ice-free means, with a range of sampling frequency (N = 1–13).

The PIRLA database (Charles et al. 1989) contains concentration data for operationally defined Al species, including "total Al," "total monomeric Al," and "organic monomeric Al" (Driscoll 1985; McAvoy et al. 1992). We obtain "inorganic monomeric Al" (= "labile monomeric Al") as the difference of the two monomeric fractions. In this paper, we refer to these environmental variables as total Al and monomeric Al and express their concentrations as micromoles per litre. For the diatom dataset, inorganic monomeric Al is the variable we evaluate because it is considered to be the active toxic fraction of Al. For the larger chrysophyte dataset, data on the organic monomeric fraction were incomplete, and consequently, we evaluate total monomeric Al. The chrysophyte dataset contains only drainage lakes, and these typically have a high proportion of their monomeric Al in the inorganic fraction (Cumming et al. 1992a).

Numerical Analysis

We use canonical correspondence analysis (CCA) (ter Braak 1986, 1987, 1988a) to explore relationships between modern diatoms and lake water chemistry (arithmetic mean values) and to assess which environmental variables can be reconstructed from fossil diatom assemblages. The approach adapted is to rationalize a set of variables that we believe a priori may explain variance in the diatom data, and then test which variables are making a significant contribution. The 13 variables in the rationalized subset are aerated pH, total alkalinity, sum of base cations, conductivity, DOC, chloride, dissolved silica, sulphate, ammonium, nitrate, total Al, inorganic monomeric Al, and Secchi transparency. Secchi transparency was included as a proxy for trophic status, as measurements of the latter were not available. Because CCA involves multiple linear regression between species assemblage scores and environmental variables, all explanatory variables, with the exception of pH and Secchi transparency, were $\log_e (x + 1)$ transformed prior to analysis due to their skewed distributions (Zar 1984). Total alkalinity was transformed as $\log_e (x + 1 + |\text{largest negative value}|)$. In the CCA, species scores are scaled to be weighted means of the sample scores, and thus the chemical variable biplot scores are correlations of the chemical variables with the axes (ter Braak 1990b).

The forward selection option in CANOCO 3.10 (ter Braak 1990a, 1990b) was used to help identify the minimal number of explanatory variables that could satisfactorily explain the variance observed in the diatom data. In order to determine whether these environmental variables could be reconstructed from fossil assemblages, we examined their relative explanatory power and independence. This was done using constrained CCA ordinations on single environmental variables, before and after removing the effect of covariables from the dataset (ter Braak 1988b). Unrestricted Monte Carlo permutation tests of each selected variable were performed to assess significance.

To analyze fish presence/absence in lakes, we added nominal variables for both fish presence (1/0) and fish absence (1/0) and then passively analyzed these new variables to find their centroids on the ordination diagram (ter Braak 1988a). The resulting centroids are single points representing the average diatom assemblages for the two classes: lakes containing fish and lakes without fish.

In addition to analyzing modern diatom assemblages and fish status in relation to lake water variables, it is possible to add fossil "passive" samples into the plane formed by the CCA axes using transition formulae (ter Braak 1988a) to form trajectories of fossil samples from scores in the modern CCA space (Birks et al. 1990a; Battarbee 1991).

Quantitative paleolimnological inferences (or reconstructions) of past environmental variables are performed in two steps (Birks et al. 1990b). In the regression step, taxon responses to the environmental variable of interest are modelled and the taxon's optimum and tolerance along the environmental gradient are estimated numerically. The second step is calibration, and it can be subdivided. First, the algal assemblage in each surface-sediment sample is used to infer the environmental variable for that sample based on the estimated taxon parameters, and the relationship between predicted and observed environmental values quantified for the entire set of "active" samples (the surface sediment assemblages with associated environmental data) to provide some assessment of predictive power such as the root mean squared error (RMSE) of prediction. Second, "passive" samples (either sediment core samples, or surface sediment samples with no associated environmental data) are used to obtain inferred environmental variable values, based on their taxa having the same characteristics as obtained for the "active" taxa in the regression step.

Weighted averaging regression (ter Braak and Looman 1986, 1987) was used to estimate monomeric Al weighted average optima (abundance weighted means) and tolerances (weighted standard deviations) for the diatom and chrysophyte taxa. Weighted averaging calibration (ter Braak and Barendregt 1986; ter Braak and van Dam 1989) with (chrysophytes), and without (diatoms) weighting taxa inversely by their squared tolerance, was used to infer monomeric Al for the modern and fossil data. $\text{Log}_e (x + 1)$ monomeric Al was used throughout. Inverse regression (Birks et al. 1990b) was used to deshrink the weighted averaging estimates for diatoms. All computations were implemented by the programs WACALIB 2.1 (Line and Birks 1990) and 3.0 (J. M. Line, C. J. F. ter Braak, and H. J. B. Birks, unpublished). For further details of weighted averaging regression and calibration, see ter Braak and van Dam (1989) and Birks et al. (1990b).

Following Birks et al. (1990b), a bootstrap root mean squared error of prediction ($\text{RMSE}_{\text{boot}}$) was used to provide error estimates of our calibration techniques. This was done because "apparent" RMSE always underestimates the "true error" (Oksanen et al. 1988; ter Braak and van Dam 1989; Birks et al. 1990b). Unique $\text{RMSE}_{\text{boot}}$ are obtained for each fossil sample that consists of (1) an estimate of prediction error unique for each sample (s_{i1}) and (2) a constant that captures the error due to the natural variation of different modern algal

assemblages occurring at the same chemistry or lakes with the same assemblages having different chemistry (s_2) (see Birks et al. 1990b for details of error estimation by bootstrapping).

Results and Discussion

Ordination of the Modern Diatom/Environmental Data

The eigenvalues for CCA axes 1 (0.80) and 2 (0.62) using all 13 chemical variables are similar or larger than those for an unconstrained detrended correspondence analysis of the diatom data (0.88, 0.36). The species–environment correlations for CCA axes 1 (0.98) and 2 (0.92) are high. We thus conclude that the measured lake water chemical variables account for the major gradients in the diatom data. In this CCA, axes 1 and 2 account for 21.9% of the variance in the species data and 45.0% of the variance in the weighted averages of the 149 diatom taxa with respect to the 13 chemical variables. This seemingly small amount of variance explained in the species data is typical of complex data sets containing many zero values (75% of the elements of the diatom data are zero). As ter Braak (1988a) commented, an ordination that explains only a low percentage can still be informative. The statistical validity of CCA axes is best assessed not by the percentage of variance explained, which is dependent on the number of variables included, but by significance tests (ter Braak 1988a). Monte Carlo permutation tests (99 unrestricted permutations of axis 1, 99 unrestricted permutations of axis 2 with axis 1 as a covariable) show that both axes are significant ($p = 0.01$ for both axes 1 and 2).

We used forward selection of environmental variables in CCA (ter Braak 1990b) to find as small a set of lake water chemical variables as possible that would explain the diatom data nearly as well as the full chemical set. In CCA with forward selection, only four variables made significant ($p \leq 0.05$, 99 unrestricted permutations) individual contributions to explaining the diatom gradients: pH (24% of the explained variance); monomeric Al (14%), Secchi (13%), and DOC (7%). The eigenvalues of axes 1 (0.78) and 2 (0.55) and species–environment correlations for axes 1 (0.97) and 2 (0.88) were only slightly lower than for a CCA using all 13 chemical variables. Both axes are significant ($p = 0.01$). We conclude that the four-variable CCA model is a reasonable representation of the major gradients in the diatom data (Fig. 1). The CCA of the diatoms spread the taxa along the four chemical gradients (Fig. 1A). The length and position of the environmental biplot arrows indicate that pH, and highly correlated variables not shown in Fig. 1A, were the most important contributors to axis 1. Generally opposite to high pH is high monomeric Al. Four lakes at the lower right have high concentrations of DOC, along a gradient that is negatively correlated with water transparency (Secchi depth) and unrelated to the pH gradient along which most of the lakes are differentiated from one another (Fig. 1B). In the ordination of the lakes (Fig. 1B), fishless lakes are located at the right side of the CCA plot, and they generally have low pH. Most fishless lakes have low DOC and high Al, although some are small seepage lakes with high DOC and midrange monomeric Al values.

We tested the strength and independence among the four significant chemical variables in a series of CCA and partial CCA runs (ter Braak 1988b) (Table 2). When CCA is run with a single environmental variable, there will be one constrained

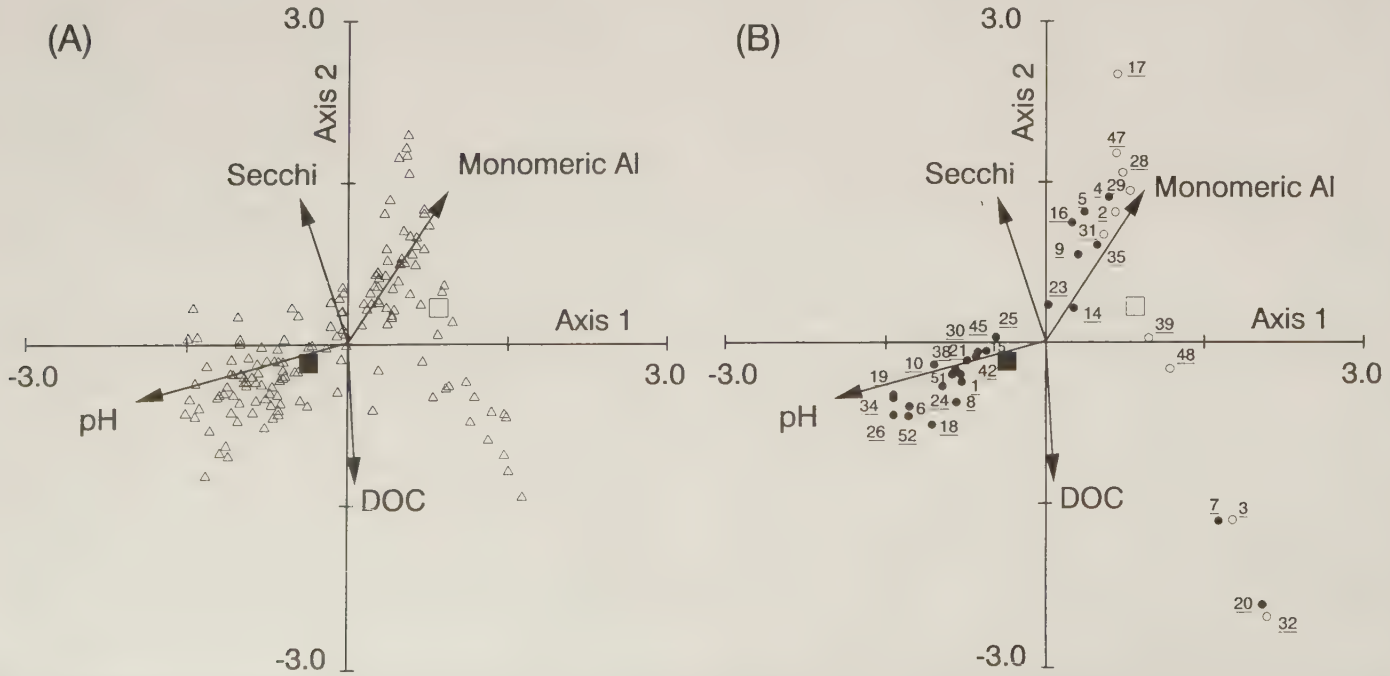

FIG. 1. Canonical correspondence analysis ordination plots of (A) 149 diatom species in 37 Adirondack lakes and (B) 37 lake surface-sediment samples, each with environmental biplot arrows for the four significant variables as determined in forward selection. Individual diatom taxa are open triangles, and diatom species assemblages are solid circles (lakes with fish) and open circles (lakes without fish). Arrows indicate the contribution of measured environmental variables to the plotted axes, with arrow tips representing high-magnitude values and the origin representing mean values for the entire dataset. Each arrow also represents an environmental gradient, with the position of diatom assemblages based on weighted mean estimates of species optima along each gradient. The two squares are the centroids of fish-containing (solid) and fishless (open) lakes. They represent average diatom assemblages for lakes in each fish resource class. Identifying lake numbers are given in Table 1 and are taken from the map in Charles et al. (1990). In the scaling used, the chemical biplot scores, here multiplied by 2, reflect the correlations of the chemical variables with the axes.

axis related to the variable included and a series of unconstrained axes. The more important an environmental variable is in explaining the variation in the biological data, the larger the first constrained axis (λ_1) will be compared with the first unconstrained axis (λ_2). For our data the largest λ_1/λ_2 ratio is for pH (Table 2), but interestingly the second largest ratio is for monomeric Al, followed by Secchi depth and DOC. For all variables the first constrained axis is significant ($p = 0.01$, 99 unrestricted Monte Carlo permutations tests). In reconstructing chemical variables from paleolimnological data, it is essential that the first constrained eigenvalue be large compared with the other eigenvalues (ter Braak 1988a). Our results suggest that both pH and monomeric Al can be reconstructed, with a considerable degree of confidence, from paleolimnological data.

To test whether monomeric Al is acting as a significant explanatory variable for the diatom data independent of variables such as pH or DOC, we partialled out the effects of the other chemical variables one at a time, as well as all possible combinations, in a series of partial CCAs and tested the significance of the monomeric Al effect with unrestricted Monte Carlo permutation tests (Table 2). In all cases the monomeric Al signal is significant ($p = 0.01$, 99 permutations). We thus conclude that there is a significant contribution from monomeric Al in explaining the observed variations in the diatom data and that this contribution is independent of the effects of pH, DOC, and Secchi depth transparency.

In all the CCA analyses, monomeric Al has a significant and stronger signal than total Al, although they have similar positions in CCA plots based on all 13 chemical variables. This is to be expected for acidic lakes from a geologically sensitive region like the Adirondack Mountains, where much of the total Al is in the toxic monomeric Al fraction (Driscoll et al. 1984; Driscoll and Newton 1985).

Core Trajectories

We investigated the trends of past lake chemistry for the four lakes by plotting diatom-based CCA trajectories for dated sediment cores (Fig. 2). The samples were positioned within the plane of CCA axes 1 and 2 on the basis of their fossil diatom composition, using transition formulae (ter Braak 1988a) to derive sample scores for the fossil-as-passive samples. All fossil samples have a low squared residual chi-square distance (3–10) (ter Braak 1990b) to axes 1 and 2 compared with the modern samples (1–14), suggesting that the positioning of the fossil samples and their trajectories in Fig. 2 is unlikely to be misleading geometrically.

Big Moose Lake became more acidic and had major increases in toxic monomeric Al between 1953 and 1982, as evidenced by the core diatom assemblages moving up and to the right (Fig. 2A); this corresponds with the observed fishery decline in Big Moose Lake since the 1940s (Schofield and Driscoll 1987). The diatom assemblage trajectory moves from being near the centroid of fish-containing lakes to pass the centroid of fishless lakes in the 1950s to 1960s. Big Moose Lake may retain more fish species than smaller lakes because of its complex watershed containing some relatively well-buffered streams (Rudd 1987; Uutala 1990), and the remaining fish species are the more tolerant ones.

Deep Lake (Fig. 2B) starts very near the fishless centroid, although the *Chaoborus* evidence and historical records indicate that fish were present before 1940 (Uutala 1990). After the early 1950s, it acidified further, gained monomeric Al, and moved past the fishless centroid.

Upper Wallface Pond (Fig. 2C), which never had fish according to the *Chaoborus* evidence, has early diatom assemblages indicating high levels of monomeric Al and consequently poor fishery potential. Its trajectory changes less than the previous two lakes; its baseline conditions are more acidic with higher monomeric Al estimates. The diatom assemblage diverges from background ca. 1900, matching the early acidification shown by Charles et al. (1990). Alternative explanations to that of toxic Al limiting fish species in Upper Wallface Pond concern its geographic setting as a high-elevation headwater lake. Upstream migration of fish to the lake may be inhibited. Furthermore, this lake may lack exposed sand or gravel needed for successful spawning of many fish species.

The diatom assemblages, and hence the past water chemistry, of Windfall Pond (Fig. 2D) have changed very little during 140 yr, and it has always had diatom assemblages strongly characteristic of fish-containing lakes with low monomeric Al.

Calibrations and Reconstructions of Monomeric Al

The summary statistics of monomeric Al concentrations in the study lakes that were used to develop the diatom and chrysophyte monomeric Al inference models are in Table 3, and the results of the diatom calibration for the monomeric Al training set are presented in Fig. 3. The statistics of the predictive

TABLE 2. Results of canonical correspondence and partial canonical correspondence analyses designed to test the strength of the four chemical variables that are significant in forward selection in CCA and the independence of monomeric Al when the effects of the other variables are partialled out. The eigenvalues (λ_1 and λ_2) represent the constrained and unconstrained variation in the biological data; see text for further details. Significance (p) was based on 99 unrestricted Monte Carlo permutation tests.

Canonical correspondence analysis				Partial canonical correspondence analysis				
Variance	λ_1	λ_1/λ_2	p	Variable	Covariable	λ_1	λ_1/λ_2	p
Monomeric Al	0.52	0.60	0.01	Monomeric Al	pH	0.44	0.62	0.01
pH	0.76	0.93	0.01		Secchi	0.52	0.68	0.01
Secchi depth	0.42	0.52	0.01		DOC	0.50	0.63	0.01
DOC	0.40	0.47	0.01		pH, Secchi	0.44	0.77	0.01
					pH, DOC	0.44	0.84	0.01
					Secchi, DOC	0.51	0.66	0.01
					pH, Secchi, DOC	0.44	0.85	0.01

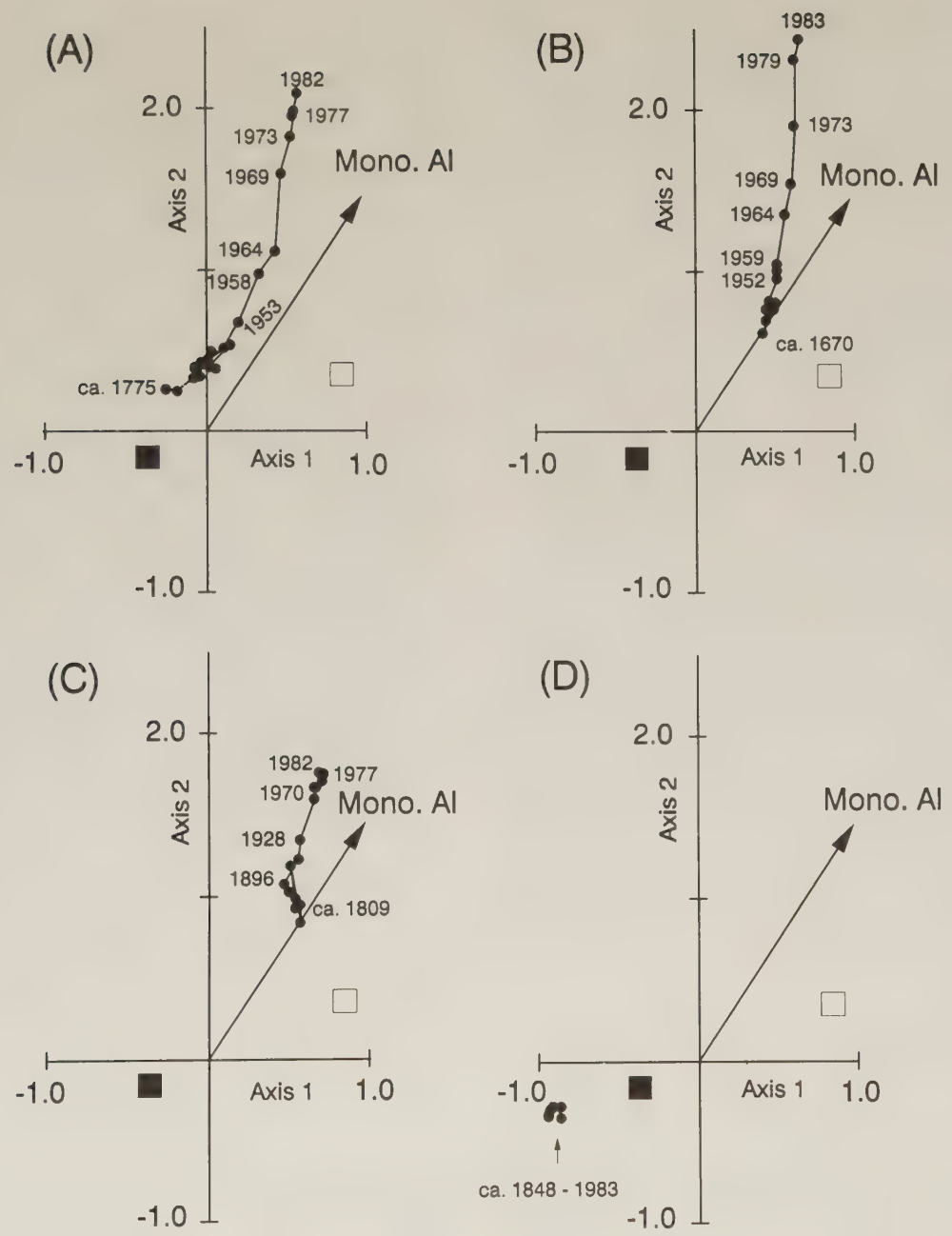

FIG. 2. Trajectory of past diatom assemblages in ^{210}Pb-dated sediment cores from four lakes in the Adirondack Park, New York. These trajectories can be superimposed onto the ordination plot in Fig. 1, with the same 2× exaggeration of biplot arrows. (A) Big Moose Lake, core 2; (B) Deep Lake; (C) Upper Wallface Pond; (D) Windfall Pond.

TABLE 3. Details of the diatom and chrysophyte calibration training sets for observed monomeric Al. The ordinations and reconstructions are based on $\log_e$ (monomeric Al + 1) values.

Training set	Range	Mean	Median	Standard deviation	Number of lakes	Number of taxa
Diatoms, monomeric Al (μmol·L^{-1})	0.0–10.7	2.17	0.60	3.01	37	149
Chrysophytes, monomeric Al (μmol·L^{-1})	0.0–14.8	2.7	0.85	3.5	62	23

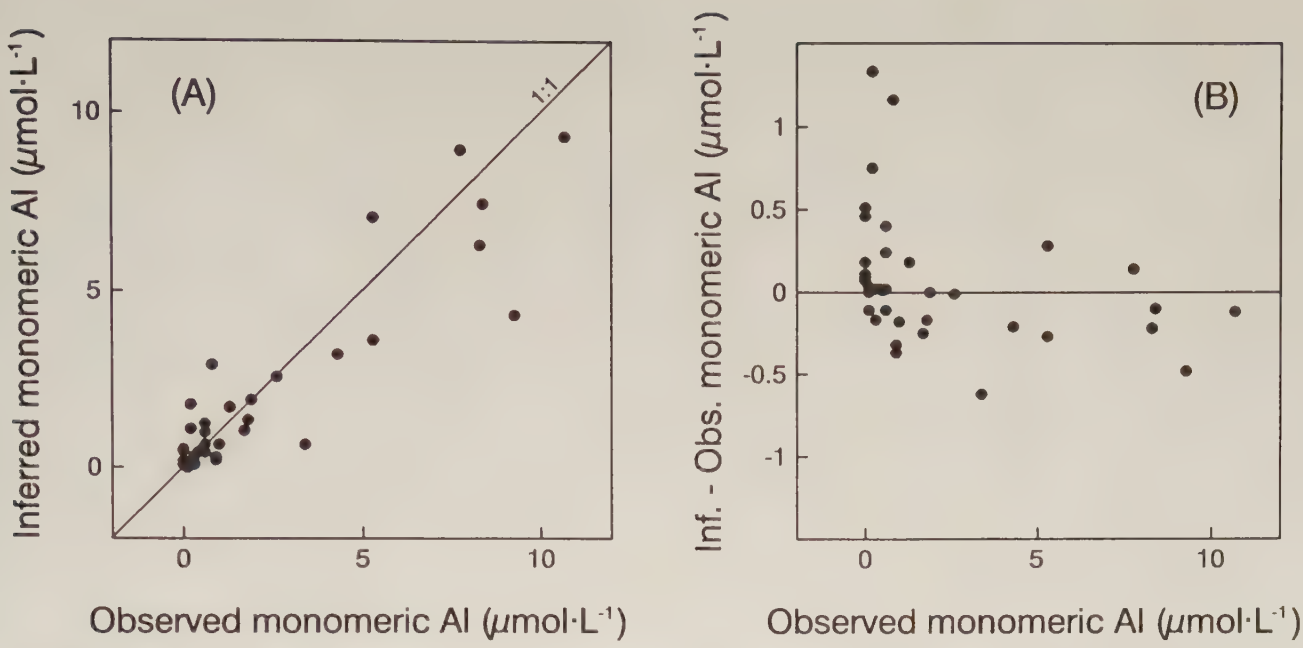

FIG. 3. Calibration relationships for the Adirondack region diatom dataset described in Table 4. (A) Inferred versus observed monomeric Al; (B) residuals versus observed monomeric Al. Data have been back-transformed from the logarithms that were used in developing the models.

TABLE 4. Summary statistics for the Adirondack diatom and chrysophyte inference models of monomeric Al. The models are based on a 37- and 62-lake "training set" for diatoms and chrysophytes, respectively. With the exception of the squared correlation coefficient, the results are presented as back-transformations of the $\log_e$ values of concentrations ($\mu mol \cdot L^{-1}$) that were used in developing the models. The chrysophyte model includes downweighting each taxon inversely by its squared tolerance (weighted averaging with tolerance downweighting, or WA(tol)) whereas the diatom model is based on estimations of optima only (simple weighted averaging, or WA). This decision was based on validation exercises carried out on WA and WA(tol) models on both the modern diatom and chrysophyte datasets.

Training set	Apparent RMSE[a]	$RMSE_{boot}$	$RMSE_{s_{i1}}$	$RMSE_{s_2}$	r^2_{adj}
Diatom	0.42	0.68	0.18	0.63	0.80
Chrysophyte	0.56	0.63	0.20	0.60	0.70

[a]RMSE = root mean squared error; see text for details.

abilities of the training sets (Table 4) show that the s_{i1} error component is considerably smaller than s_2 suggesting that the training sets are adequate to yield estimates of species parameters by WA regression required for WA calibration. However, the relationships between observed and inferred values (Fig. 3) show a trend such that high observed values tend to be underestimated and lower values overestimated. This is particularly apparent in the plots of residuals versus observed values (Fig. 3). A similar trend is present in a total Al calibration in Europe (Birks et al. 1990a). This trend in the residuals plotted against observed values probably results from functional interactions between pH and monomeric Al (Birks et al. 1990a). Such interactions are not accounted for in simple WA inferences of individual variables.

The diatom- and chrysophyte-based reconstructions of monomeric Al (Fig. 4) fit well with the historic fisheries information (Schofield and Driscoll 1987), with *Chaoborus*-based paleolimnological assessments (Uutala 1990), and with paleolimnological assessments of lake acidification (Charles et al. 1990).

Previously, Kingston and Birks (1990) have shown that DOC concentrations in Big Moose Lake decreased during lake acidification, but the magnitude of change is small. Monomeric Al concentrations in Big Moose Lake increased dramatically by the early 1950s, and may have stabilized by the early 1980s (Fig. 4A) to the late 1980s (Sullivan et al. 1992; Dixit et al. 1992). Although Big Moose Lake has retained a few fish populations, the magnitude and timing of reconstructed Al trends correspond well with known extirpation of several species, including smallmouth bass and brook trout. Our reconstructed concentrations are, however, too high by a factor of 1.4, compared with the measured values (ice-free means). This may be attributed to the diatom assemblage of core 2 not having close surface analogues in the training set (the relative abundance of taxa with high-magnitude Al optima was greater in the core than it was in surface sediments from the same lake collected separately). The core-top diatom assemblage is nearly identical to that from surface sediments at Upper Wallface Pond (pH = 4.96, monomeric Al = 5.3 $\mu mol \cdot L^{-1}$), as is the chemistry (Big Moose Lake, pH = 5.08, monomeric Al = 5.3 $\mu mol \cdot L^{-1}$), but Upper Wallface Pond is fishless. Our inaccuracy in reconstructing the magnitude of monomeric Al in 1982 may be related to the dynamic nature of Al species in lake water (Driscoll et al. 1984; Schafran and Driscoll 1987), so that the annual mean concentration value which we have used may not adequately represent the conditions affecting the biological response variables. Seasonal and episodic events and microhabitat variations have not been integrated into the broad scale of paleolimnological analyses (Pillsbury and Kingston 1990). Furthermore, spring values (during high flow) of monomeric Al are approximately twice as high as the mean values (T. J. Sullivan, E&S Environmental Chemistry, Inc., P.O. Box 609, Corvallis, OR 97339, USA, pers. comm.), and we suspect that further analyses using the higher spring values would provide an even stronger biological response signal. The chrysophyte

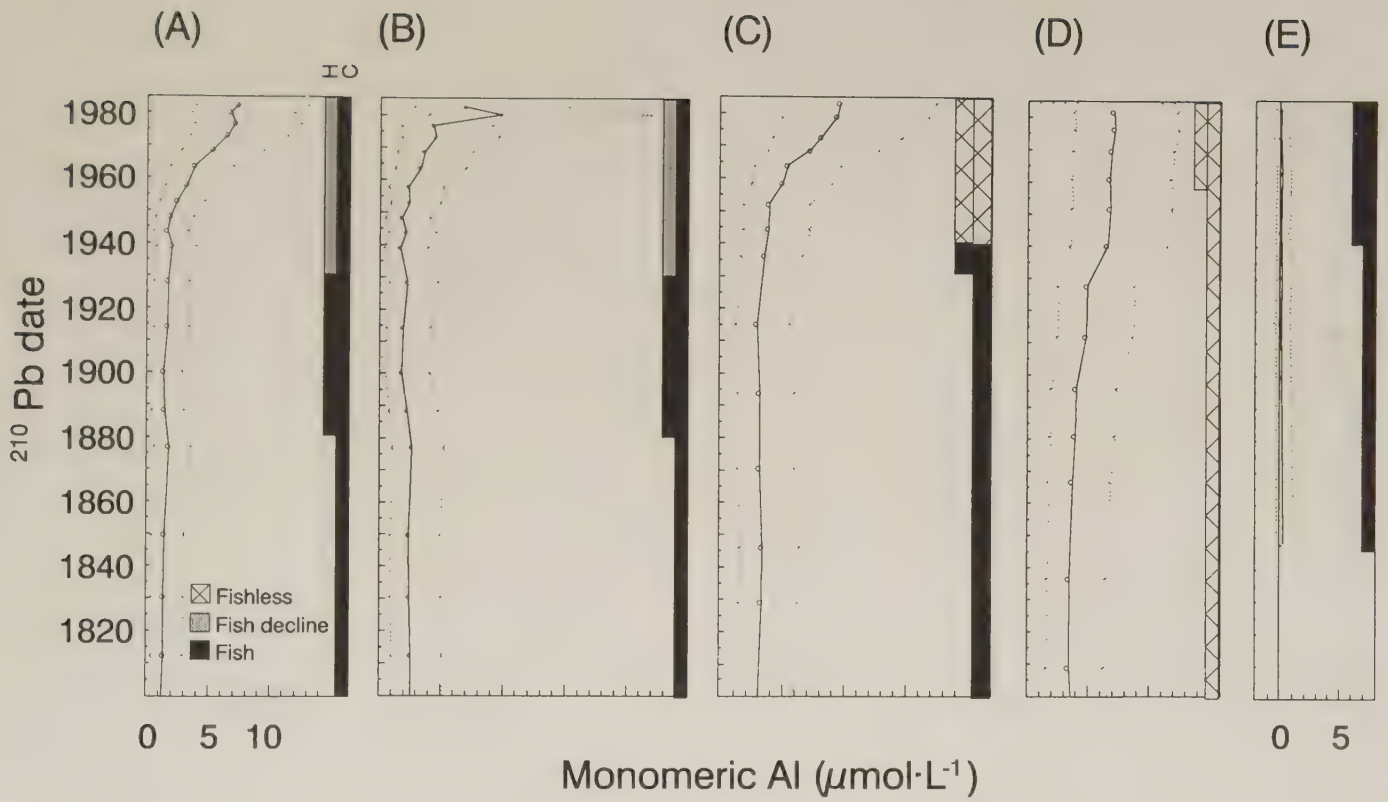

FIG. 4. Reconstructions of (A) diatom-based monomeric Al and (B) chrysophyte-based monomeric Al for Big Moose Lake and diatom-based monomeric Al for (C) Deep Lake, (D) Upper Wallface Pond, and (E) Windfall Pond. Reconstructions are bounded by bootstrapping estimates of the root mean squared error of prediction for each sample (see text for further details). Bars to the right of each reconstruction indicate historical (H) and *Chaoborus*-based (C) reconstructions of fishery resources. The historical fish records are not continuous, unlike the paleolimnological records. Intervals older than ca. 1840 are dated by extrapolation, and their ages are not considered to be reliable (Binford 1990).

reconstruction of monomeric Al generally agrees with the diatom reconstruction in timing, but a higher background and recent concentration are indicated (Fig. 4B). This discrepancy may be related to the life strategies of these two algal groups and/or the larger errors associated with the chrysophyte inferences. The scaled chrysophytes occur mainly in the spring, and are therefore more likely to show pH decreases and Al increases associated with snowmelt (Cumming et al. 1992b).

Deep Lake, which apparently lost its fish ca. 1940 (Uutala 1990), had its largest increase in monomeric Al after 1950, but a slight increase in the 1940s is evident (Fig. 4C). Deep Lake was naturally acidic (pH $\leq$ 5.5), but had a major recent acidification at the same time as the major Al increase (Charles et al. 1990). The background monomeric Al concentration was perhaps twice as high as Big Moose Lake, indicating that the fish populations were potentially stressed even before industrial deposition began.

Upper Wallface Pond never had fish, according to the *Chaoborus* evidence (Uutala 1990). This pond is naturally acidic and has acidified further recently, but it started acidifying earlier than most other Adirondack Park Lakes (ca. 1900, Charles et al. 1990). It is possible that fish were never able to migrate to this lake, which is in the High Peaks Wilderness Area of the Park, and that suitable spawning substrates are not available. However, our reconstructions provide further insight as to why fish were never there. Upper Wallface Pond has even higher background monomeric Al (~4 μmol·L^{-1}, Fig. 4D) than Deep Lake, and its natural background Al concentrations were perhaps too toxic to allow fish survival. Monomeric Al began increasing ca. 1900, with the biggest increase in the early 1930s. This scenario matches well with toxicity discovered in field and laboratory studies (Baker and Schofield 1980; Schofield and Driscoll 1987; Driscoll and Schecher 1988; Holtze and Hutchinson 1989). For example, Holtze and Hutchinson (1989) found that fry and "eyed eggs" of several fish species were adversly affected below pH 5.1, with total Al of 3–7 μmol·L^{-1}. The paleolimnological evidence indicates that the predeposition fishery potential of Upper Wallface Pond was much less than that of Big Moose Lake and Deep Lake.

Windfall Pond is circumneutral, has not acidified (Charles et al. 1990), and has always had fish (Uutala 1990). Our reconstruction of past monomeric Al (Fig. 4E) shows that it has always been near 0 μmol·L^{-1}, which is to be expected based on the less acidic water chemistry.

Comparison with Other Estimates of Al

Paleolimnological reconstructions of monomeric Al provide new information which can be compared with Al trends and magnitudes modelled by other means (e.g. MINEQL, Charles 1984; Charles et al. 1987; ALCHEMI, Sullivan et al. 1989; empirical relationship between Al and pH, Sullivan et al. 1990). Charles et al. (1987) modelled total Al increases in Big Moose Lake of ~15 μmol·L^{-1} between 1850 and 1982. This matches our reconstruction fairly well (Fig. 4A), when one considers that we are reconstructing only part of this total.

Sullivan et al. (1990) give an empirical relationship for estimating monomeric Al in Adirondack drainage lakes from

TABLE 5. Observed, present-day inferred, and pre-1850 inferred inorganic monomeric Al concentrations based on the direct diatom relationship in this paper compared with values inferred by an empirical relationship between monomeric Al and pH (Sullivan et al. 1990) using the pH reconstructions from Charles et al. (1990, table 3). Units are $\mu mol \cdot L^{-1}$.

Lake	Observed "calibration" monomeric Al	Recent (1982) diatom inferred[a]	Recent (1982) from empirical relationship	Pre-1850 diatom inferred[a]	Pre-1850 from empirical relationship
Big Moose Lake	5.3	7.4 (6.9)	4.2	1.1 (2.0)	1.2
Deep Lake	10.7	9.4	9.5	2.7	2.4
Upper Wallface Pond	5.3	7.1	5.4	3.9	2.8
Windfall Pond	1.0[b]	0.12	0.9	0.3	0.3

[a]Chrysophyte inference is shown in parentheses.
[b]RILWAS data from the outlet stream, approximately 1.5 km downstream from Windfall Pond.

autumn lake water pH. Our four study lakes are drainage lakes with paleolimnologically inferred pH values (Charles et al. 1990), so we can compare our inferred monomeric Al concentrations with estimated values based on pH (Table 5). The reconstructed concentrations for recent Big Moose Lake and Upper Wallface Pond and for pre-1850 Upper Wallface Pond (Fig. 4) are larger than the values obtained from chemical regression methods whereas the paleolimnologically reconstructed concentration for Windfall Pond is smaller than that obtained by the empirical relationship.

Current ideas about the geochemistry of Adirondack region lakes suggest that such high reconstructed preindustrial monomeric Al concentrations would be unlikely, unless some unspecific source of mineral acids could be rationalized. However, considering the strong and predictable effect that Al has on diatoms and chrysophytes in the Adirondack datasets, we propose that Al concentrations in some lakes have been higher than previously deduced from chemical data and modelling alone.

Conclusions

Monomeric Al provides a significant signal in explaining algal response variables, and lake water Al concentration has increased predictably during lake acidification of sensitive Adirondack Park lakes. The Al signal can be evaluated using diatoms and/or chrysophytes, and using different subsets of Adirondack Park PIRLA data.

Aluminum toxicity is known at many trophic levels, and hypothesized mechanisms for microalgae include interference with nutrient uptake and/or metabolism (Nalewajko and Paul 1985; Gensemer 1990; Smith 1990). Such mechanisms of algal toxicity provide the link that allows evaluation of fisheries status using algae.

Although this paper does not use modern passive samples, we could make the same sort of assessment for annually integrated algal collections from lake sediments or sediment traps, for any new lake of interest in the Adirondack Mountains. This is exactly what will be required of biological data in long-term monitoring programs.

The reconstructed chemical changes in Big Moose Lake, Deep Lake, and Upper Wallface Pond during this century can now be seen to indicate Al increases, not just pH decline, in that the core trajectories closely follow the monomeric Al gradient. Charles (1984) and Charles et al. (1987, 1990) demonstrated this using traditional stratigraphies and noted the increase in species from high-Al lakes, but the CCA trajectories in Fig. 2 are more conclusive. Such trajectories have been used previously to show changes during lake acidification (Battarbee et al. 1988; Birks et al. 1990a; Battarbee 1991).

The timing of major excursions in Al concentration matches the known history of fishery declines and the *Chaoborus*-based paleolimnological assessments with known dating errors (SD = ~4 yr). Furthermore, we find that Al increases have continued in the study lakes past the date of declining sulphate deposition and stabilization of combustion markers such as PAH (ca. 1970) (Furlong et al. 1987; Charles et al. 1990).

Although presence/absence type of fish data are easiest to obtain and suited to our analytical techniques, it is possible to use ranked or continuous data which may provide a more detailed evaluation of fish populations. Studies at more than one level of the foodweb (e.g. Johnson and Wiederholm 1989; this study) are quite feasible and should be pursued to obtain a broader understanding of ecosystem function.

Canonical correspondence analysis and weighted averaging regression and calibration provide useful tools for evaluating past trends in lake water Al and fish stocks. The additional benefits of increased autecological knowledge obtained with these techniques will improve future paleolimnological research, and should be transferrable from acidification to other research areas including long-term monitoring.

Finally, our techniques could be used to verify independent evaluations of fishery resources (e.g. Kelso et al. 1990), by using statistically selected subpopulations of lakes to project to broader regional scales.

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How Much Acidification Has Occurred in Adirondack Region Lakes (New York, USA) since Preindustrial Times?

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Preindustrial and present-day lake water pH, acid neutralizing capacity (ANC), total monomeric aluminum (Al_m), and dissolved organic carbon (DOC) were inferred from the species composition of diatom and chrysophyte microfossils in the tops (present-day inferences) and bottoms (pre-1850 inferences) of sediment cores collected from a statistically selected set of Adirondack lakes. Results from the study lakes were extrapolated to a predefined target population of 675 low-alkalinity Adirondack region lakes. Estimates of preindustrial to present-day changes in lake water chemistry show that approximately 25–35% of the target population has acidified. The magnitude of acidification was greatest in the low-alkalinity lakes of the southwestern Adirondacks, an area with little geological ability to neutralize acidic deposition and receives the highest annual average rainfall in the region. We estimate that ~80% of the target population lakes with present-day measured pH $\leq$ 5.2 and 30–45% of lakes with pH between 5.2 and 6.0 have undergone large declines in pH and ANC, and concomitant increases in [Al_m]. Estimated changes in [DOC] were small and show no consistent pattern in the acidified lakes. This study provides the first statistically based regional evaluation of the extent of lake acidification in the Adirondacks.

Le pH, la capacité de neutraliser l'acidité (CNA), la teneur en aluminium total monomère (Al_m) et la teneur en carbone organique total dissous (COD) de l'eau de lacs des Adirondack ont été déterminés, tant pour la période pré-industrielle que pour la période actuelle, à partir de la composition spécifique des diatomées et chrysophytes fossiles de carottes de sédiments prélevées dans un groupe de lacs déterminé statistiquement. La partie supérieure des carottes correspond à l'époque actuelle et la partie inférieure à celle d'avant 1850. Les résultats obtenus pour les lacs étudiés ont été extrapolés à une population cible pré-définie de 675 lacs faiblement alcalins de la région des Adirondacks. Les variations estimées des paramètres chimiques de l'eau entre l'époque pré-industrielle et l'époque actuelle indiquent que de 25 à 35 % environ des lacs de la population cible se sont acidifiés. L'acidification a été plus importante chez les lacs faiblement alcalins du sud-ouest des Adirondacks, zone qui a peu de capacité géologique de neutraliser les dépôts acides et où la moyenne annuelle des précipitations est la plus élevée de la région. Nous avons estimé que 80 % environ des lacs de la population cible dont le pH actuel est de 5,2 ou moins et que de 30 à 45 % des lacs à pH se situant entre 5,2 et 6,0 ont subi d'importantes baisses du pH et de la CNA accompagnées d'augmentations des teneurs en Al_m. Les variations estimées du COD sont faibles et ne présentent pas de tendance cohérente dans les lacs acidifiés. L'étude constitue la première évaluation (basée sur des statistiques) régionale de l'étendue de l'acidification des lacs dans les Adirondacks.

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Acidic deposition continues to be a major ecological issue. This is especially true for the Adirondack Mountain region, a large forested area located in northern New York State (USA) underlain primarily by granitic gneiss and metasedimentary rocks. This region receives elevated inputs of sulphate (SO_4^{2-} deposition approximately 550 $eq \cdot ha^{-1} \cdot yr^{-1}$) and nitrate ($NO_3^-$ deposition approximaely 370 $eq \cdot ha^{-1} \cdot yr^{-1}$) (Driscoll et al. 1991). The combination of geological materials with little ability to neutralize acidic deposition and large inputs of strong acids places aquatic resources in this region at risk (e.g. Driscoll et al. 1991). According to Phase I of the U.S. Environmental Protection Agency (USEPA) Eastern Lake Survey (ELS-I), the Adirondack region contains the largest number of acidic lakes in the northeast: 138 (11%) of the 1290 lakes >4 ha in surface area in the Adirondacks are currently acidic (defined as acid neutralizing capacity (ANC) ≤ 0 $\mu eq \cdot L^{-1}$) and 459 lakes (36%) have ANCs ≤ 50 $\mu eq \cdot L^{-1}$ (Brakke et al. 1988). Many Adirondack lakes show the effects of acidic deposition (e.g. Schofield 1976; Schofield and Driscoll 1987; Sullivan 1990; Charles et al. 1990; Driscoll et al. 1991), but until now an accurate regional assessment of the number of acidified lakes (i.e. those that have lost ANC) has not been made. Such an assessment of regional changes in lake water chemistry is of great interest to both scientists and policy makers.

Historical changes in water chemistry have been assessed by (1) comparing historical chemical data with current measurements, (2) assessments of past water chemistry based on empirical geochemical and/or mechanistic lake–watershed models, and (3) paleolimnological studies. All three approaches have inherent strengths and weaknesses and therefore should be used in a collaborative fashion.

Comparison of historical and current measurements of ANC or pH has the potential to provide the most direct evidence for lake acidification. Such studies have consistently indicated recent acidification of some Adirondack lakes (e.g. Schofield 1982; Kramer et al. 1986; Asbury et al. 1989, 1990; Kramer 1990; Metcalf and Gerlach 1990), although they differ in the magnitude and extent of the inferred acidification. The main differences among these studies concern the comparability of data, since the alkalinity titration endpoints used in the historical surveys were inadequately documented and remain a source of controversy (Asbury et al. 1989, 1990; cf. Kramer et al. 1986; Kramer 1990). In addition, the earliest historical data were collected in the 1930s or later, often after the onset of acidic deposition, and data do not exist for many low-alkalinity lakes of particular interest (Sullivan 1990).

Computer models have been adapted for hindcasting past water chemistry (e.g. Wright et al. 1986; Jenkins et al. 1990). One problem with this approach is that the mechanisms regulating surface water acidification are numerous, partially interdependent, and incompletely understood (Marmorek et al. 1989; Turner et al. 1990). Because it is impossible for models to include the detailed mathematical representations of all hydrological and biogeochemical processes that affect acidification in individual watersheds and lakes (Turner et al. 1990), it is difficult to assess the accuracy of model-based hindcasts of historical change (Sullivan et al. 1990a, 1992).

Paleolimnological studies using biological remains of diatoms (Bacillariophyceae) and scaled chrysophytes (Chrysophyceae and Synurophyceae) in sediment cores can provide quantitative reconstructions of historical water chemistry, provided adequate calibration sets are available (Battarbee et al. 1990; Birks et al. 1990b). Previous paleolimnological studies in the Adirondacks were designed to document changes in individual lakes (e.g. Del Prete and Schofield 1981; Charles 1984; Smol et al. 1984a, 1984b; Smol 1986; Charles and Norton 1986; Uutala 1986, 1990; Davis et al. 1988; Charles et al. 1990). These studies show that many of the currently acidic lakes have acidified since preindustrial times, but since these lakes were not statistically chosen, we do not know how representative these inferences are for Adirondack lakes in general.

Given the uncertainties in historical water chemistry data, model hindcasts, and the small number of paleolimnological studies, we undertook a statistically designed paleolimnological study (part of the Paleoecological Investigation of Recent Lake Acidification-II (PIRLA-II) project; Charles and Smol 1990) in the Adirondack region to (1) provide regional inferences of the preindustrial (pre-1850) lake water chemistry and (2) estimate the extent, magnitude, and spatial patterns of preindustrial to present-day changes in lake water chemistry. These inferences are based on the extrapolation of paleolimnological results from a set of statistically chosen lakes to a predefined target population of 675 relatively undisturbed, low-alkalinity Adirondack region lakes (see Methods for details).

Methods

This study is built on the methods, quality assurance, and quality control (QA/QC) procedures (Charles and Whitehead 1986), database management system (Charles et al. 1989), and taxonomic documentations (Camburn et al. 1984–86; Camburn and Kingston 1986; Charles 1986) established during the PIRLA-I project, as well as the statistical inference methods developed during the Palaeolimnology Programme of the Surface Water Acidification Project (SWAP) (Birks et al. 1990a, 1990b; Kingston and Birks 1990). Minor additions and modifications from the PIRLA-I protocols are detailed in Smol et al. (1989).

Rationale

Present-day (0- to 1-cm interval) and pre-1850 (>20 cm, usually >25 cm) sediment core intervals were analyzed for both diatom and chrysophyte microfossils from a set of statistically selected Adirondack region lakes (see Lake Selection and Study Site Characteristics). ^{210}Pb profiles from over 25 Adirondack sediment cores indicate that the preindustrial (ca. 1850) sediment intervals are usually located below 20 cm (Cumming 1991). In addition, previous studies (Charles et al. 1990), as well as replicate cores presented in this paper, suggest that paleolimnological inferences of water chemistry from sediment core intervals below 20 cm were relatively stable in preindustrial times. Diatom-based and chrysophyte-based inferences of preindustrial and present-day lake water chemistry were made with transfer functions that use the relative abundances of the taxa in a sample to infer water chemistry (see Water Chemistry Inference Methods and Error Estimation Techniques). Differences between present-day and preindustrial water chemistry values provided estimates of historical changes in lake water chemistry. These estimates were then extrapolated to the target population of Adirondack lakes. Inferences of pH and total monomeric aluminum (Al_m) were inferred from both diatoms and chrysophytes whereas only diatoms were used to infer ANC and dissolved organic carbon (DOC).

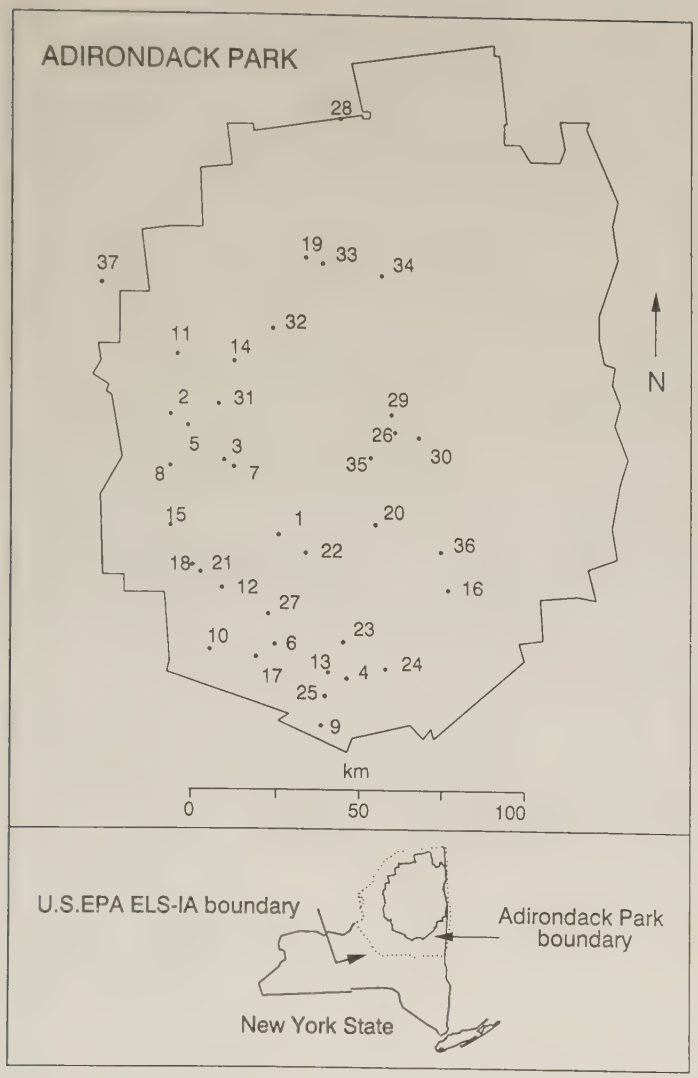

FIG. 1. Location of the 37 Adirondack study lakes. See Table 1 for lake and pond names.

Lake Selection and Study Site Characteristics

Over 760 lakes in the northeastern United States were sampled in Phase I of the Eastern Lake Survey (ELS-I) during the fall of 1984 (Linthurst et al. 1986; Kanciruk et al. 1986; Landers et al. 1988). These lakes were chosen, systematically with a random start, from the lakes identifiable on U.S. Geological Survey 1:250,000-scale maps, after they had been stratified into five subregions based on physiographic features, and three mapped strata based on surface water ANC. The ELS subregion 1A is one of the five subregions sampled by the ELS-I and encompasses most of northern New York State including the Adirondack Park (Fig. 1). The 38 study lakes used here were selected as probability samples from a subset of ELS-I subregion 1A lakes by the USEPA Direct/Delayed Response Project (DDRP) (Church et al. 1989). This subset of ELS lakes represents a "target population" of relatively undisturbed, low-alkalinity (ANC $< 400\ \mu eq \cdot L^{-1}$) Adirondack region lakes with maximum depths >1.5 m, surface areas between 4 and 200 ha, and watershed areas <3000 ha. For each of the 38 lakes, a known probability of inclusion was calculated based on the statistical design; the inverse of the inclusion probability is the weighting factor. The weighting factor gives the number of lakes in the target population represented by each study lake (Table 1); extrapolation to the target population is based on the weighting factors. For a more comprehensive coverage of lake selection procedures, see Church et al. (1989). Two of the original 38 study lakes were not included in the extrapolation of our results to the target population: Oxbow Lake was eliminated because it was not possible to remove a sediment core from this lake, as the sediments were completely covered by macrophytes (Cumming et al. 1989), and Nick's Pond was eliminated because it had been limed in 1983 prior to being sampled by the ELS-I. The liming and earlier water chemistry data suggested that Nick's Pond was not representative of the high ANC ELS-I lakes for which it was sampled. The other lakes that have been limed <5 yr before sediment coring and/or water chemistry measurements (Table 2) were not eliminated from this study because the effects of the liming were thought to be minimal. Extrapolation to the target population was therefore based on weighting factors from the remaining 36 study lakes; the target population therefore represents 675 lakes (i.e. the sum of the weighting factors for the 36 lakes).

All of the study lakes are located within the boundary of the Adirondack ecological zone; and all but one within the Adirondack Park (Fig. 1). With the exception of two seepage lakes, all of the study lakes were drainage lakes (Table 1). The lakes range in elevation from 227 to 792 m and generally have small watersheds (Table 1). Lake type and disturbance variables were deduced from maps, aerial photographs, and limited field reconnaissance (Tables 1 and 2). Evidence of liming was obtained from records at the New York State Department of Environmental Conservation.

Field and Laboratory Procedures

Sediment cores were taken from the deepest basin of each lake using a modified Kajak–Brinkhurst gravity corer (Glew 1989) equipped with a Plexiglas tube with an internal diameter of 6.5 cm. On the lake shore, the cores were sectioned into 1-cm intervals or less using a close-interval sediment sectioner (Glew 1988). Replicate cores were taken for backup purposes.

In the laboratory, homogeneous aliquots of sediment from the top (0- to 1-cm interval; present-day sediment) and the bottom (>20 cm, usually >25 cm; pre-1850 sediment) of the primary core from each study lake were digested in a solution of sulphuric acid and potassium dichromate. The resulting suspensions were centrifuged and washed with distilled water until the material was acid free (Smol 1983). An aliquot of each suspension was then diluted with distilled water and poured into evaporation trays (Battarbee 1973), where the microfossils were allowed to settle onto microscope coverslips. Once dry, the coverslips were mounted on glass slides using Hyrax®. Four slides were produced for each sample processed.

At least 500 diatom valves and chrysophyte scales were counted along transects under oil immersion (1000–1250× magnification, NA = 1.3 or 1.4) from one prepared slide per sample. K. E. Camburn counted the diatom valves and B. F. Cumming the chrysophyte scales. Chrysophyte taxonomic references included Asmund and Kristiansen (1986), Kling and Kristiansen (1983), Nicholls (1982), Siver et al. 1990, Smol et al. (1984a), Takahashi (1978), and Wee (1982), as well as J. P. Smol's reference collection. Diatom photographs and taxonomic references and designations were based primarily on Camburn et al. (1984–86), Camburn and Kingston (1986), and Charles (1986). Quality assurance design and implementation

TABLE 1. Lake morphometry and Direct/Delayed Response Project (DDRP) statistical weighting factors for the Adirondack PIRLA-II study lakes. The weighting factors were calculated by the DDRP (Church et al. 1989). They give the number of lakes represented by each sampled lake from the defined target population of lakes (see text for further details). The lakes are arranged in order of increasing present-day measured pH, as in Table 2 and Fig. 6 and 7.

Lake name	ELS ID[a]	Map No.[b]	Hydrologic type[c]	Elevation (m)	Lake area (LA)[d] (ha)	Maximum depth (m)	Watershed area (WA)[d,e] (ha)	WA/LA	DDRP weight[d]
Wolf Lake	1A1-061	1	D	792	4	13	184	47	12.29
Middle South Pond	1A1-049	2	D	605	19	3	114	6	12.29
Gull Lake (South)	1A1-073	3	DO	594	10	4	108	10	12.29
Duck Lake	1A2-004	4	D	704	11	6	212	20	12.29
Hawk Pond	1A1-003	5	D	645	13	10	80	6	12.29
Trout Lake	1A2-054	6	D	651	17	9	220	13	12.29
Constable Pond	1A1-017	7	D	582	19	4	888	48	12.29
Hitchcock Lake	1A1-057	8	DO	567	15	14	125	9	12.29
No Name (Mud Pond)	1A2-048	9	DO	485	5	11	41	9	12.29
Curtis Lake	1A3-028	10	S	393	5	12	13	6	12.29
Nick's Pond	1A1-038	11	D	515	6	7	38	6	40.47[f]
South Lake	1A3-065	12	D	615	196	18	1375	7	12.29
Chub Lake	1A2-052	13	DO	504	7	6	55	8	12.29
John Pond	1A1-039	14	DO	546	6	8	38	7	12.29
Grass Pond	1A3-048	15	D	546	5	5	260	53	12.29
St. John Lake	1A2-002	16	D	673	13	11	138	11	12.29
North Branch Lake	1A2-042	17	D	575	8	8	172	22	12.29
Woodhull Lake	1A1-066	18	D	572	399	27	1383	4	12.29
Dry Channel Pond	1A1-028	19	D	496	25	4	151	6	12.29
Long Pond	1A3-046	20	D	565	14	13	134	10	22.49
Fourth Lake	1A1-020	21	D	609	23	9	266	12	12.29
Whitney Lake	1A1-012	22	D	749	40	12	219	5	12.29
Mud Lake	1A2-041	23	D	527	7	3	318	45	22.49
Woods Lake	1A2-045	24	D	418	26	12	149	6	12.29
Nine Corner Lake	1A2-046	25	D	570	42	9	165	4	12.29
Cheney Pond	1A3-042	26	D	503	8	4	142	19	22.49
Wilmurt Lake	1A1-014	27	D	752	37	4	209	6	22.49
Lake Frances	1A2-006	28	S	454	6	7	114	19	22.49
Zack Pond	1A3-040	29	DO	566	36	10	202	6	22.49
Nate Pond	1A3-001	30	D	614	8	6	83	11	22.49
Partlow Lake	1A1-046	31	D	534	31	6	346	11	22.49
Mt. Arab Lake	1A1-064	32	DO	506	51	17	217	4	22.49
Middle Pond	1A1-029	33	D	484	24	3	125	5	40.47
Kiwassa Lake	1A1-033	34	D	466	109	13	391	4	40.47
Unknown Pond	1A3-043	35	D	497	14	9	462	34	40.47
Fish Ponds (NE)	1A2-037	36	D	486	8	4	819	109	40.47
Trout Lake	1A2-058	37	D	227	149	24	455	3	40.47

[a]ELS ID = Eastern Lake Survey identification number (Linthurst et al. 1986).
[b]Map No. corresponds to those shown on Fig. 1.
[c]S = seepage, DO = drainage (outlet only), D = drainage (Bernert and Sullivan 1990).
[d]DDRP Adirondack data (Church et al. 1989).
[e]Does not include lake area.
[f]Nick's Pond was improperly assigned to the high-ANC cluster and was therefore not used in any extrapolations to the target population (see text for further details).

is documented in Charles and Whitehead (1986), Kreis (1989), Cumming et al. (1990), and Kingston et al. (1992b).

Multiple-Core Lakes

To test the assumption that water chemistry reconstructions from a single deepwater sediment core are reproducible, the top and bottom intervals of triplicate cores from four Adirondack lakes were analyzed. All triplicate cores were obtained from the deepest basin (within 80% of the maximum depth) of each study lake (Cumming et al. 1989). Three of these lakes are low-alkalinity lakes (ANC <25 $\mu eq \cdot L^{-1}$) and show a wide range of basin depth and complexity (Constable Pond, maximum depth = 4 m; Woods Lake, maximum depth = 12 m; and South Lake, maximum depth = 19 m) whereas the fourth lake, Clear Pond, is a deep (maximum depth = 24 m) higher alkalinity lake (ANC = 110 $\mu eq \cdot L^{-1}$). Diatom and chrysophyte preparation and enumeration follow the methods outlined in the previous section, except that only 100 chrysophyte scales per sample were counted for Clear Pond because scales were not abundant in this lake.

Water Chemistry Inference Methods and Error Estimation Techniques

The paleolimnological transfer functions used here are derived from the relationship between surface-sediment diatom and chrysophyte assemblages and measured lake water chemistry from 71 Adirondack drainage lakes (the training or calibration set). These lakes include the drainage lakes with maximum depths >2 m used in Charles and Smol (1988) in addition to the PIRLA-II drainage lakes used here. The sediment samples were collected in 1979 or the early 1980s (Charles 1985) and in 1987–88 (Cumming et al. 1989) from the deepest part of the lake basins using either a modified Cushing and Wright (1965) piston corer or a modified Kajak–Brinkhurst

TABLE 2. Selected mean lake water chemistry and watershed disturbance data for the 37 Adirondack PIRLA-II study lakes. Water chemistry are arithmetic averages taken from a number of sources (see text for details).

Lake	pH (aerated)	ANC ($\mu eq \cdot L^{-1}$)	Al_m (μM)[a]	Total Al ($\mu g \cdot L^{-1}$)	DOC (μM)	Cabin count[b]	Liming[c]	Beaver lodge count[c]
Wolf Lake	4.4	−39	14.8	718	531	0	0	0
Middle South Pond	4.5	−27	7.7	320	199	0	0	0
Gull Lake (South)	4.6	−15	10.0	425	278	0	0	0
Duck Lake	4.6	−17	10.6	510	207	0	0	2
Hawk Pond	4.7	−14	10.0	425	278	0	0	0
Trout Lake	4.8	−9	5.4	314	400	0	0	1
Constable Pond	4.8	−7	7.2	420	354	0	0	0
Hitchcock Lake	4.9	−9	7.5	416	498	0	0	2
No Name (Mud Pond)	5.1	−4	1.9	141	446	0	0	0
Curtis Lake	5.1	−6	0.5	29	262	1	1	0
Nick's Pond[f]	5.2	−1	—	59	—	0	2	0
South Lake	5.3	4	1.8	158	143	26	0	1
Chub Lake	5.3	5	3.6	260	415	0	0	0
John Pond	5.3	2	3.0	122	101	0	0	1
Grass Pond	5.4	7	5.9	359	345	0	0	1
St. John Lake	5.4	3	2.0	115	143	1	1	0
North Branch Lake	5.5	7	3.4	293	399	2	1	0
Woodhull Lake	5.5	4	1.4	82	155	20	0	1
Dry Channel Pond	5.6	6	1.1	173	305	7	0	2
Long Pond	5.8	27	0.3	32	247	0	0	0
Fourth Lake	5.9	17	3.1	203	347	0	2	0
Whitney Lake	6.0	13	0.7	45	204	0	0	0
Mud Lake	6.1	22	1.0	131	548	0	0	0
Woods Lake	6.2	15	0.5	34	170	0	0	0
Nine Corner Lake	6.3	18	0.4	113	161	0	2	0
Cheney Pond	6.4	23	1.0	93	346	0	0	0
Wilmurt Lake	6.7	40	0.4	188	182	8	2	0
Lake Frances	6.7	40	0.2	32	339	0	0	0
Zack Pond	6.8	80	0.1	27	205	7	0	0
Nate Pond	6.8	80	0.5	50	354	0	0	0
Partlow Lake	6.9	68	0.7	293	425	1	1	0
Mt. Arab Lake	6.9	95	0.3	13	212	37	0	0
Middle Pond	7.0	110	0.4	102	538	0	0	0
Kiwassa Lake[d]	7.3	205	0.4	13	312	59	0	0
Unknown Pond[d]	7.4	276	0.4	63	537	0	0	1
Fish Ponds (NE)	7.4	170	0.4	116	463	2	0	4
Trout Lake	7.8	396	0.3	33	314	137	0	0

[a]Total monomeric aluminum.
[b]Direct/Delayed Response Project data (Church et al. 1989) with minor additions by PIRLA-II coring crew.
[c]0 = no liming record, 1 = limed more than 5 yr before coring data and/or water chemistry, 2 = limed <5 yr before coring and/or water chemistry (Bernert and Sullivan 1990).
[d]Potentially influenced by road salt, i.e. a major road upstream or a major road within 40 ft. of lake elevation (Bernert and Sullivan 1990).
[e]Determined by DDRP from aerial photos (Church et al. 1989) with minor additions by PIRLA-II coring crew.
[f]Water chemistry for Nick's Pond is from preliming conditions (Charles et al. 1990); this lake was improperly assigned to the high-ANC cluster and was therefore not included in any of the population estimates (see text for further details).

TABLE 3. Summary of the water chemistry samples used to calibrate the diatom and chrysophyte inference models (see text for details).

Variable	Mean number of samples per lake	Median number of samples per lake	Range of samples per lake	Number of calibration lakes
pH	5.1	4	1–14	71
Acid neutralizing capacity (ANC)	4.9	4	1–14	71
Total monomeric aluminum (Al_m)	3.2	2	1–13	62
Dissolved organic carbon (DOC)	3.1	2	1–14	71

corer (Glew 1989). More information on the location, morphometry, and chemistry of individual lakes is available elsewhere (Church et al. 1989; Cumming et al. 1989; Charles et al. 1990).

The number of lakes used to calibrate each diatom and chrysophyte inference model depended on the available measured water chemistry in the 71 lakes because not all chemistry was available for all lakes (Table 3). Calibration water chemistry

TABLE 4. Summary statistics of the Adirondack diatom and chrysophyte inference models for pH, total monomeric aluminum (Al_m), acid neutralizing capacity (ANC), and dissolved organic carbon (DOC). Water chemistry inference models were developed using weighted-averaging regression and calibration techniques (see text for further details).

Model	Number of lakes	r^2	Standard error	Apparent RMSE[a]	$RMSE_{boot}$[b]
Diatom-inferred pH	71	0.91	0.31	0.31	0.35
Chrysophyte-inferred pH	71	0.72	0.63	0.62	0.69
Diatom-inferred $\log_e (Al_m + 1)$ (μM)	62	0.83	0.39	0.39	0.45
Chrysophyte-inferred $\log_e (Al_m + 1)$ (μM)	62	0.70	0.57	0.56	0.63
Diatom-inferred $\log_e$ (ANC + 40) ($\mu eq \cdot L^{-1}$)	70	0.90	0.27	0.26	0.31
Diatom-inferred DOC (μM)	71	0.64	85	84	123

[a]RMSE = root mean squared error.
[b]$RMSE_{boot}$ = root mean squared error after bootstrapping, a computer-intensive resampling procedure (see text for further details).

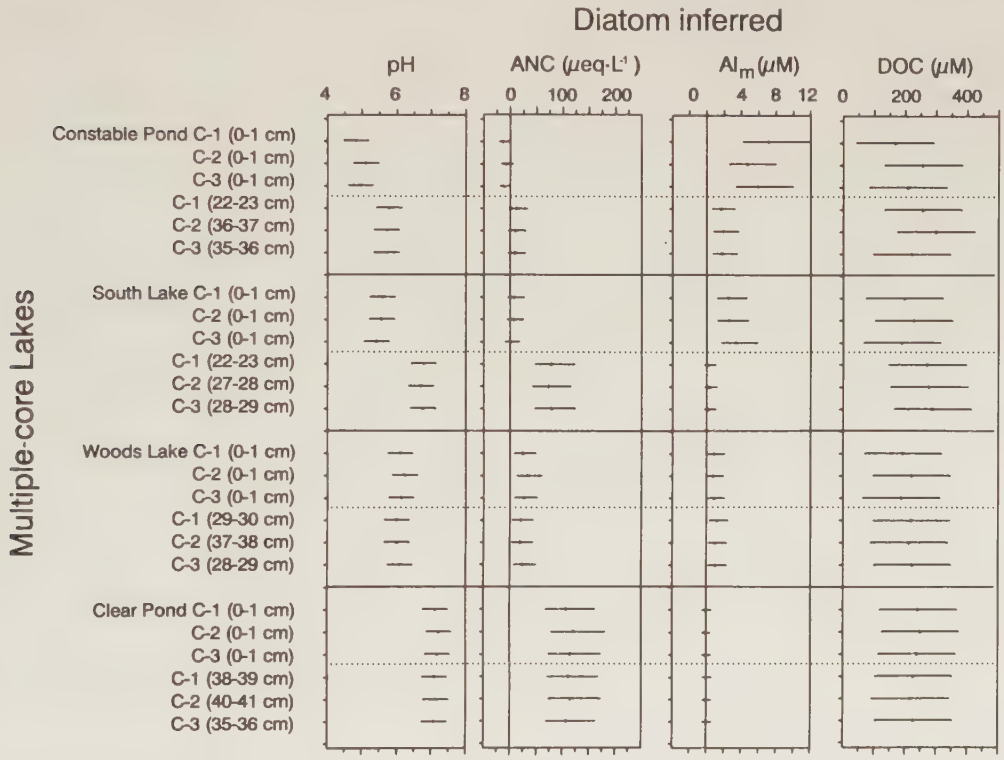

FIG. 2. Diatom-inferred pH, acid neutralizing capacity (ANC), total monomeric aluminum (Al_m), and dissolved organic carbon (DOC) from the top (0–1 cm) and the bottom (>20 cm, usually >25 cm) of triplicate cores from the replicate core lakes. Triplicate cores were obtained from the deepest basin (within 80% of the maximum depth) from each study lake. Constable Pond, South Lake, and Woods Lake are low-pH lakes (pH < 6.5) and reflect a gradient of basin depth and complexity (Table 1). Clear Pond (E.L.) is a deep, high-pH lake (pH > 7; Charles et al. 1990). The bottom interval from each core represents the lowermost interval obtained from that core. Error bars represent $\pm RMSE_{boot}$ (see text for details).

are arithmetic averages of samples collected at either 0.5 m depth in the PIRLA-I project and the Regional Integrated Lake-Watershed Acidification Study (RILWAS) (Gherini et al. 1989) and/or from 1.5 m in ELS-I and -II some unpublished water chemistry. The PIRLA water chemistry includes two to seven samples collected in the summers of 1979–82 for 30 of the calibration lakes (Charles 1985; Charles et al. 1990); RILWAS water chemistry includes 6–13 samples collected during May–September 1982–84 for seven of the calibration lakes (see Driscoll and Newton 1985). The ELS-I dataset (1984) includes a single fall measurement for 45 of the calibration lakes (see Kanciruk et al. 1986 and Linthurst et al. 1986). The ELS-II dataset includes one to three measurements for 28 of the calibration lakes collected during the fall of 1986 (USEPA Corvallis Environmental Research Laboratory). Single samples for 19 of the calibration lakes were collected in August–September 1988 and standard procedures were used to measure aerated pH, [Al_m], and [DOC] (C. Driscoll, Syracuse University, and A. Uutala,

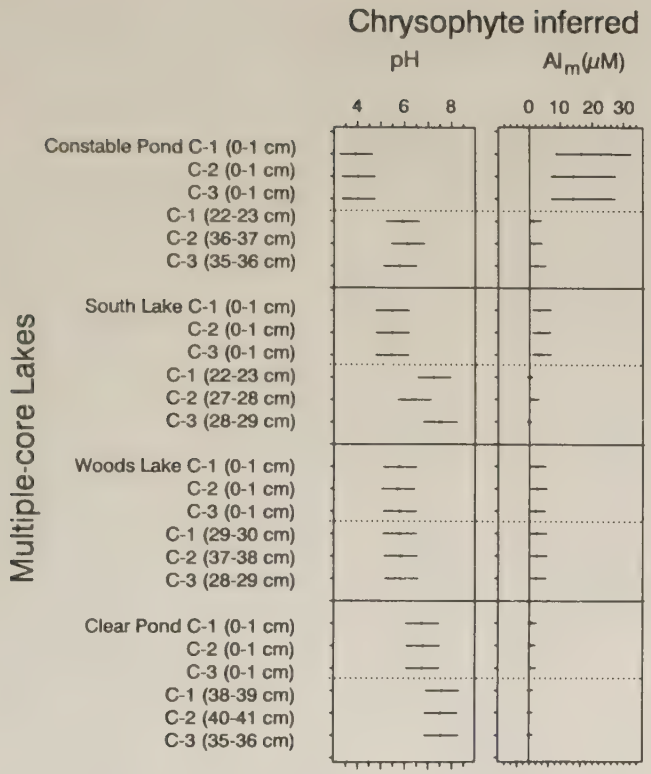

FIG. 3. Chrysophyte-inferred pH and total monomeric aluminum (Al_m) from the top (0–1 cm) and the bottom (>20 cm) of triplicate cores from the four replicate core lakes. Error bars represent $\pm RMSE_{boot}$ (see text for details). See Fig. 2 caption for details.

Queen's University). Inference models were developed for $[Al_m]$, since the inorganic fraction of $[Al_m]$ was not available from all of the calibration lakes. Nevertheless, since the inference models were developed using only drainage lakes, the majority of the $[Al_m]$ would be in the inorganic form.

Inference models were developed using weighted averaging (WA) regression and calibration techniques based on Birks et al. (1990a, 1990b) and ter Braak and van Dam (1989). Weighted averaging and associated bootstrapping were implemented using WACALIB version 3.0 (J. M. Line, C. J. F. ter Braak, and H. J. B. Birks, unpubl. program). An earlier version of this program (WACALIB version 2.1) is commercially available (Line and Birks 1990). These techniques have also been widely used in SWAP (Battarbee et al. 1990). The theory, application, and implementation of WA are discussed in ter Braak and Looman (1986), ter Braak and Barendregt (1986), ter Braak and Prentice (1988), and Line and Birks (1990).

The diatom-based and chrysophyte-based inference models were developed using WA with classical deshrinking (Birks et al. 1990b; Kingston et al. 1992a). Diatom-based inference models were developed for pH, ANC, $[Al_m]$, and [DOC] whereas chrysophyte-based inference models were developed for pH and $[Al_m]$ (Cumming et al. 1992; PIRLA-II unpublished data). The squared correlation coefficient, standard error, apparent root mean squared error (apparent RMSE), and overall bootstrap root mean squared error of prediction ($RMSE_{boot}$, based on 1000 cycles) are presented to show the predictive abilities of the inference models (Table 4). The diatom-based models have superior predictive powers than the chrysophyte-based models and therefore should be considered more reliable.

Bootstrapping is a computer-intensive resampling procedure that can be used to obtain reliable error estimates while still using the full size of the original calibration set (Efron 1983). Bootstrapping error estimates can be partitioned into (1) the prediction error (s_{i1}) involved in calculating the optima and/or tolerances of the taxa and (2) the prediction error (s_2) due to natural variation in the dataset (Birks et al. 1990b). In this study, s_{i1} generally accounted for a small proportion (<5–10%) of the overall $RMSE_{boot}$ for the present-day and pre-1850 samples. Thus, the $RMSE_{boot}$ of prediction based on the training set provides comparable and realistic error estimates for both present-day and pre-1850 inferences and was therefore used as the criterion for discussing and comparing the historical changes in water chemistry (Table 4). For details of error estimation by bootstrapping, see Birks et al. (1990b).

Results and Discussion

How Accurate Are Inferred Chemistry Values?

Inferences of water chemistry from the tops and bottoms of triplicate cores taken from four lakes show good agreement (Fig. 2 and 3). Variability among replicate preindustrial and present-day water chemistry inferences is low. Similar reproducibility was seen in the PIRLA-I project (Charles et al. 1991).

The diatom-based and chrysophyte-based water chemistry inferences show that Constable Pond and South Lake have acidified since preindustrial times whereas Woods Lake has not changed. The triplicate core inferences of water chemistry from Clear Pond differed between the diatoms and chrysophytes. The diatom-based inferences suggest that the acid/base status of this lake has remained stable since preindustrial times whereas the chrysophyte-based inferences suggest recent pH declines. This discrepancy may be related to the life strategies of these two algal groups. The scaled chrysophytes are exclusively planktonic, and because they are more abundant in the spring (Takahashi 1978; Kristiansen 1986), they are more likely to show pH decreases and $[Al_m]$ increases associated with snowmelt. In conclusion, diatom-based and chrysophyte-based inferences of lake water chemistry from the top and bottom of replicate deepwater sediment cores are reproducible. These analyses support the use of a single core to represent changes for individual lakes.

What Were Preindustrial Lake Water Chemistry Conditions?

The diatom and chrysophyte inferences of distribution of preindustrial pH and $[Al_m]$ for the target population show good overall agreement. Both models indicate that in preindustrial times there were few (~15%) naturally acidic lakes (ANC $\leq$ 0, pH $\leq$5.5), and <5% of lakes had $[Al_m] > 5$ μM (Fig. 4 and 5). None of the lakes had a diatom-inferred preindustrial pH < 5.0. [DOC] in preindustrial times did not exceed 550 μM (Fig. 4).

How Has Lake Water Chemistry Changed Since Preindustrial Times?

A larger proportion of lakes have present-day pH values <6.5 (ANC $\leq$25–50 $\mu eq \cdot L^{-1}$) and $[Al_m] > 4$ μM compared with preindustrial times (Fig. 4 and 5). Lake water [DOC] has also increased in a number of lakes since preindustrial times. Extrapolation of the diatom-based and chrysophyte-based esti-

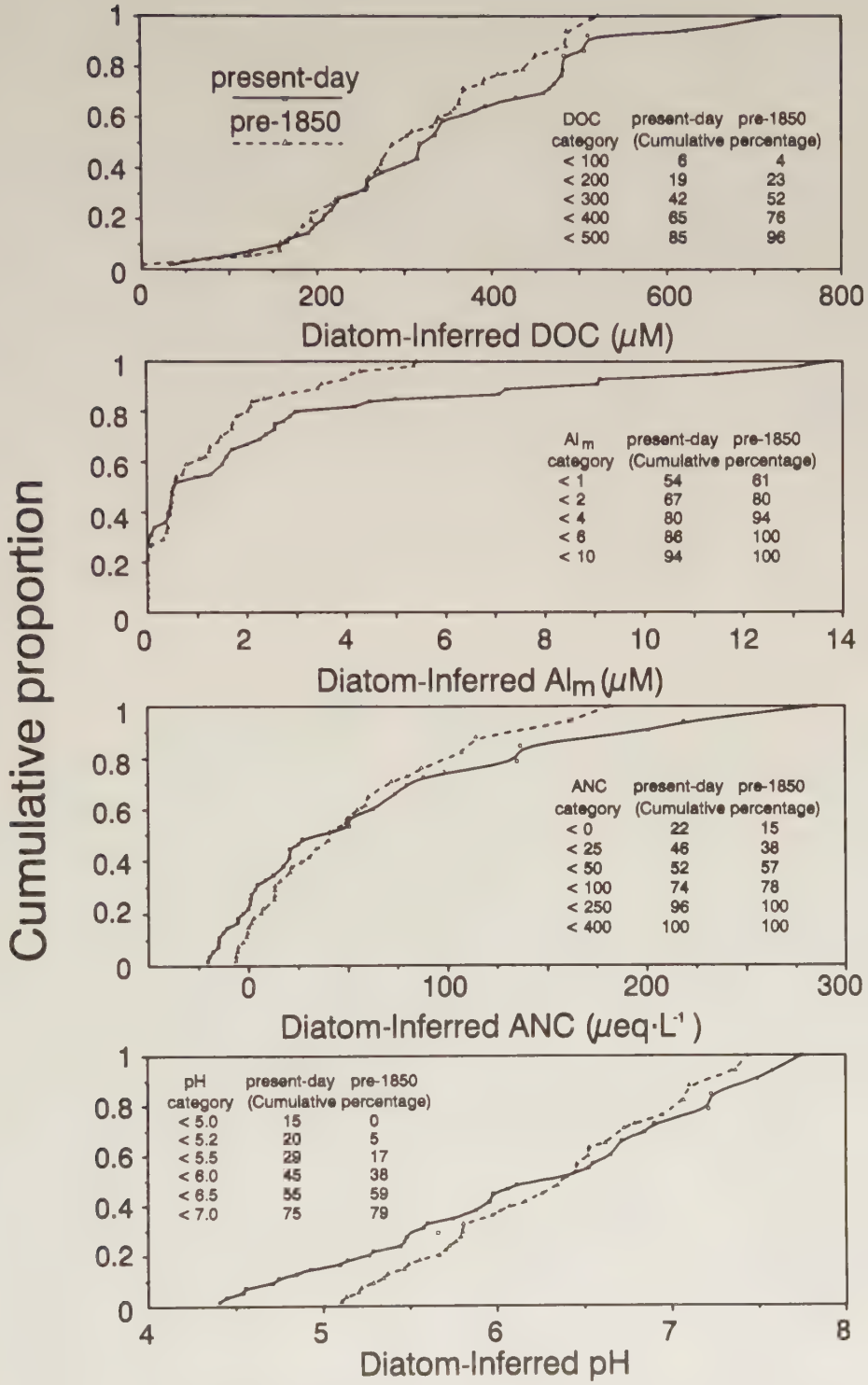
present-day
pre-1850
DOC category | present-day (Cumulative percentage) | pre-1850
< 100 | 6 | 4
< 200 | 19 | 23
< 300 | 42 | 52
< 400 | 65 | 76
< 500 | 85 | 96
Diatom-Inferred DOC (μM)
Al m category | present-day (Cumulative percentage) | pre-1850
< 1 | 54 | 61
< 2 | 67 | 80
< 4 | 80 | 94
< 6 | 86 | 100
< 10 | 94 | 100
Diatom-Inferred Alm (μM)
ANC category | present-day (Cumulative percentage) | pre-1850
< 0 | 22 | 15
< 25 | 46 | 38
< 50 | 52 | 57
< 100 | 74 | 78
< 250 | 96 | 100
< 400 | 100 | 100
Diatom-Inferred ANC (μeq·L-1)
pH category | present-day (Cumulative percentage) | pre-1850
< 5.0 | 15 | 0
< 5.2 | 20 | 5
< 5.5 | 29 | 17
< 6.0 | 45 | 38
< 6.5 | 55 | 59
< 7.0 | 75 | 79
Diatom-Inferred pH
Cumulative proportion

FIG. 4. Cumulative proportion of present-day and pre-1850 diatom-inferred distributions of pH, acid neutralizing capacity (ANC), total monomeric Al (Al_m), and dissolved organic carbon (DOC) for the target population of Adirondack lakes.

mates of change in lake water chemistry that were greater than the $RMSE_{boot}$ of prediction of the inference models to the target population shows good agreement (Table 5). Approximately 25–35% of the target population lakes have undergone declines in pH of this magnitude; most of these lakes also show large increases in $[Al_m]$. The magnitude of acidification was greatest in lakes with present-day pH < 6.0 or ANC values ≤25–50 $\mu eq \cdot L^{-1}$). Approximately 80% of the target population lakes with current pH < 5.2 and 40% of the lakes with current pH between 5.2 and 6.0 are estimated to have undergone large declines in pH and ANC and increases in $[Al_m]$ (Table 5; Fig. 6 and 7).

The magnitude of acidification was much less in lakes with present-day pH > 6 (Fig. 6 and 7; Table 5). In fact, the diatom-based estimates show that a number of lakes with pH values >6.5 increased in pH and ANC (Fig. 6). Lakes that

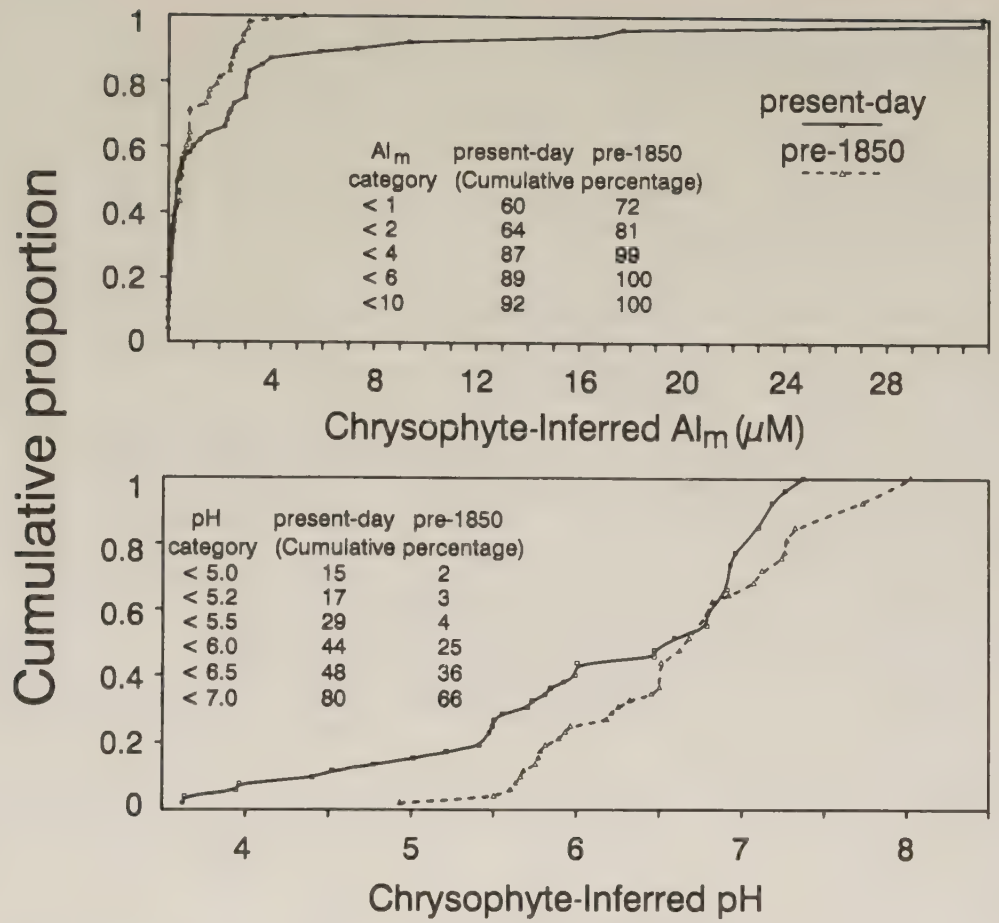

FIG. 5. Cumulative proportion of present-day and pre-1850 chrysophyte-inferred distributions of pH and total monomeric Al (Al_m) for the target population of Adirondack lakes.

TABLE 5. Percentage of lakes in the target population that have undergone declines in pH and acid neutralizing capacity (ANC), and increases in total monomeric Al (Al_m). These percentages are based on the extrapolation of the diatom and chrysophyte inferences from the study lakes that are greater than the $RMSE_{boot}$ of prediction for the different models (see Table 3). The diatom and chrysophyte inferences are based on 36 and 33 lakes, respectively, since chrysophyte remains were not abundant in three lakes (Middle South Pond, Zack Pond, Trout Lake). Since chrysophytes estimates of change were missing from three lakes, the weighting factors for the chrysophyte extrapolations became 12.88, 25.3, and 50.59 for the <25, 25–100, and 100–400 $\mu eq \cdot L^{-1}$ ANC clusters. This recalculation resulted in a slight discrepancy in the number of lakes in each of the pH classes but not in the overall target population. The results are expressed as the percentage of lakes in three pH classes based on measured pH.

	Number of lakes in target population		Percentage of lakes that have decreased in pH		Percentage of lakes that have decreased in ANC (diatom extrapolation only)	Percentage of lakes that have increased in Al_m		Percentage of lakes that have increased in DOC (diatom extrapolation only)
pH class	Diatom	Chrysophyte	Diatom	Chrysophyte		Diatom	Chrysophyte	
$pH \leq 5.2$	123	116	80	78	70	70	78	30
$5.2 < pH \leq 6$	145	154	42	33	25	34	16	8
$pH > 6$	407	405	6	19	6	6	6	14
All classes	675	675	27	33	22	24	21	16

showed increases in pH and/or ANC greater than their respective $RMSE_{boot}$ of prediction are all located at low elevations and have had land-use disturbance in their watersheds (Zack Pond, Partlow Lake, Mt. Arab Lake, and Trout Lake; Table 1). Contrary to the diatom results, the chrysophyte inferences of change show that a number of the lakes with present-day measured pH > 6 have acidified, although only two of these lakes have declined more than the $RMSE_{boot}$ of prediction. The pH declines in these lakes were accompanied by only small increases in $[Al_m]$. Alkalinity increases have been associated with watershed disturbance (Haines and Akielaszek 1983; Eilers et al. 1989) and biogeochemical reactions such as sulphate reduction (Kilham 1982; Cook et al. 1986), as well as biological assimilation of atmospherically deposited nitrogen species (Sullivan 1990). The importance of in-lake alkalinity generation to alkalinity budgets in lakes with short residence times has not been fully evaluated (Schindler 1986; Schafran and Driscoll 1987; Shaffer et al. 1988; Shaffer and Church 1989).

The lakes that acidified are located primarily in the southwestern Adirondacks (Fig. 8). Generally, these lakes are at ele-

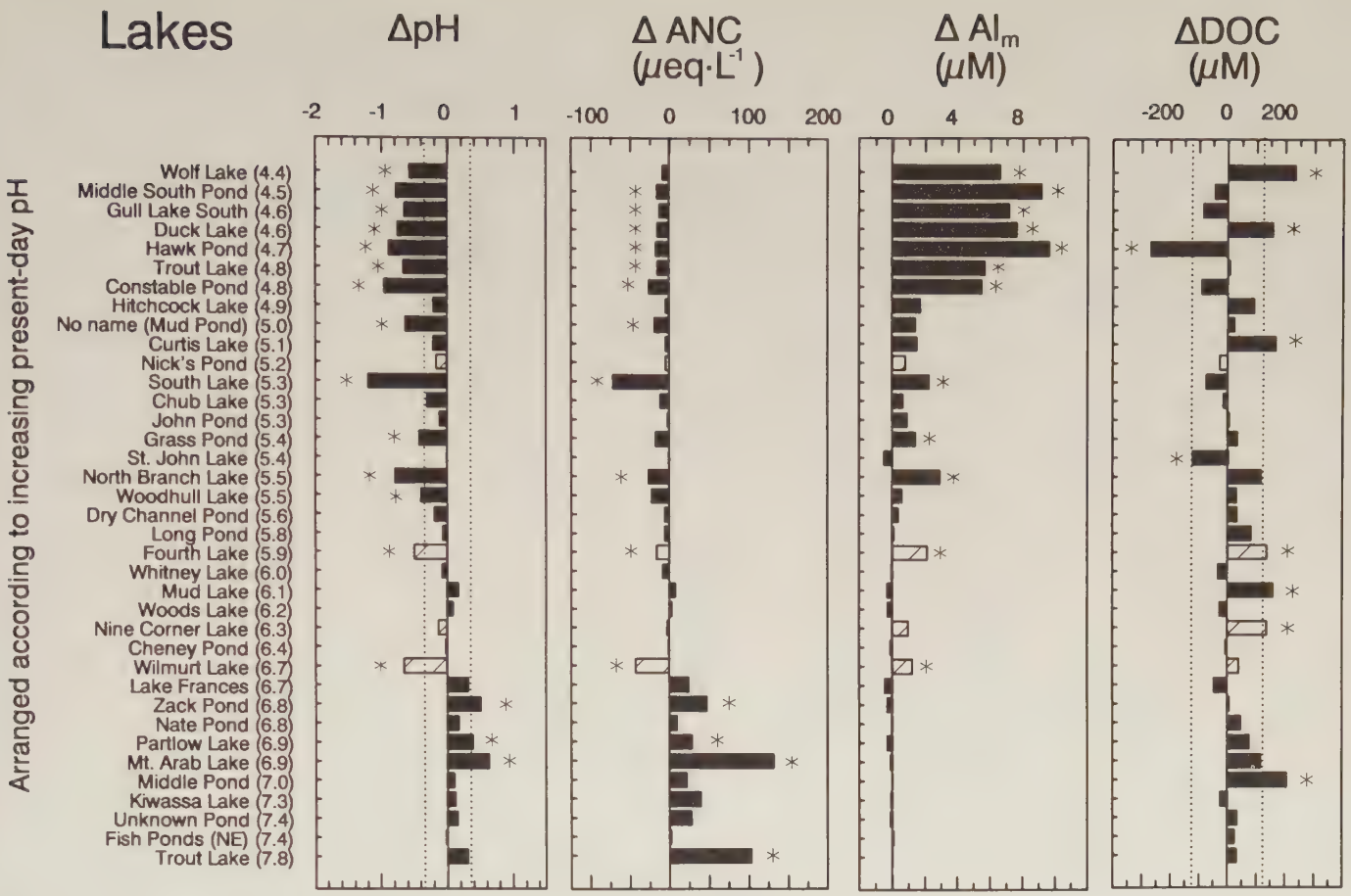

FIG. 6. Diatom-based estimates of historical change (preindustrial to the present) in pH, acid neutralizing capacity (ANC), total monomeric aluminum (Al_m), and dissolved organic carbon (DOC) from the 37 Adirondack study lakes. The estimates are presented as the differences in inferred water chemistry between the top (0–1 cm) and bottom (>20 cm, usually >25 cm) sediment core intervals. The lakes are arranged according to increasing present-day measured pH. Lakes indicated by hatched bars were limed within 5 yr before coring and/or assessment of water chemistry. The dotted lines represent $\pm RMSE_{boot}$ of prediction for pH and DOC. Estimates of change in ANC and Al_m are presented as differences between back-transformed estimates since these models were developed on $\log_e$ data; thus, simple lines for $\pm RMSE_{boot}$ cannot be presented. Asterisks denote changes that are $>RMSE_{boot}$ for the various inference models.

vations >600 m, have watersheds with thin soils overlying granitic bedrock that is relatively resistant to weathering, and receive >110 cm of precipitation per year (Sullivan et al. 1990a). These lakes typically have small watershed areas, although there is little difference in the ratio of watershed area to lake surface compared with other Adirondack lakes (Table 1). High-elevation areas that receive large amounts of precipitation would be expected to acidify rapidly because of the high load deposition of strong mineral acids on generally thinner soils.

The extent and magnitude of pH declines and [Al_m] increases indicated by the diatom-based models (Fig. 6 and 8) are generally smaller than those indicated by the chrysophyte-based models (Fig. 7 and 8), except when lakes have been limed within 5 yr of the coring date. In these instances, the diatom-based models show acidification whereas the chrysophyte-based models show pH increases. These discrepancies are probably related to the respective life strategies of diatoms and chrysophytes as explained previously. The results from the limed lakes should be interpreted cautiously because the placement of the lakes along the pH gradient may be misleading and/or the paleoestimates of change may be underestimated. The latter appears to be more of a problem with the chrysophyte estimates.

The estimated changes in lake water pH and ANC in this study indicate widespread acidification of low-alkalinity Adirondack lakes in the southwestern Adirondacks and possible episodic acidification of higher alkalinity lakes (Eshleman 1988; Wigington et al. 1990). The magnitude of acidification estimated in this study is somewhat less than computer model estimates. For example, MAGIC model (Cosby et al. 1985a, 1985b) estimates of change in lake water ANC, based on the same lakes used in this study, produced larger estimates of ANC declines than either the diatom or chrysophyte estimates (Sullivan et al. 1990a). Apparently, neutralization of acidic inputs has been more important than previously estimated (Sullivan et al. 1990b), and/or preindustrial lake water conditions were more acidic than was originally assumed.

The inferred changes in [DOC] show no consistent pattern in the acidified lakes (Fig. 6). It has been hypothesized that organic acids may have exerted a greater influence on lake water pH during preindustrial times and that [DOC] has decreased in response to increased organic acid protonation and increased concentrations of Al (Almer et al. 1974; Krug and Frink 1983; Davis et al. 1985; Krug 1989; Kingston and Birks 1990). Further research is needed regarding the response of organic acids to anthropogenic acidification.

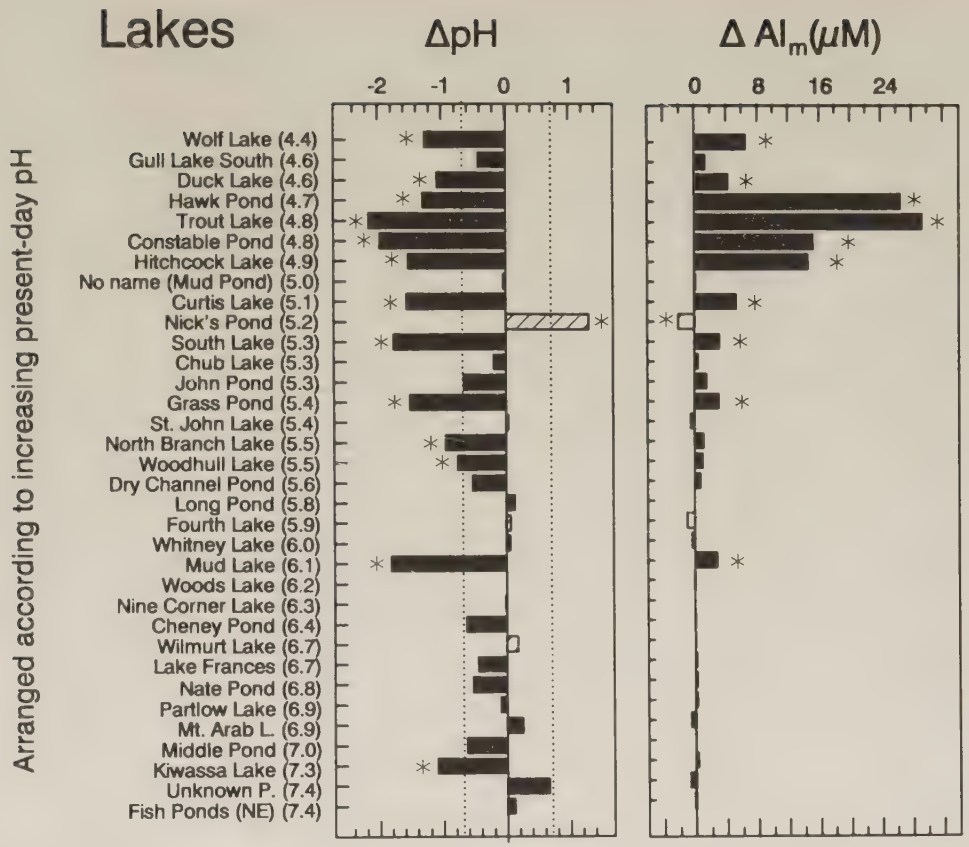

FIG. 7. Chrysophyte-based estimates of historical changes (preindustrial to the present) in pH and total monomeric aluminum (Al_m) from the 37 Adirondack study lakes presented as the differences in inferred water chemistry between the top (0–1 cm) and bottom (>20 cm, usually >25 cm) sediment core intervals. The dotted line represents $\pm RMSE_{boot}$ of prediction for pH. Please refer to Fig. 6 for common details.

Conclusions

The results presented in this paper are applicable to a target population of approximately 675 relatively undisturbed, low-alkalinity (ANC $< 400\ \mu eq \cdot L^{-1}$) Adirondack region lakes with maximum depths >1.5 m, surface areas between 4 and 200 ha, and watershed areas <3000 ha. Lakes with surface areas <4 ha were not included in this study, although they are an important resource in northern New York. These smaller lakes tend to have lower pH and ANC and higher [DOC] than lakes with surface areas >4 ha, primarily because many are seepage lakes (Kretser et al. 1989).

Based on our paleolimnological inferences, only 15% of the target population lakes were naturally acidic (ANC ≤ 0) or had $[Al_m] > 3\ \mu M$. None of the lakes had a diatom-inferred pH < 5.0, in contrast with the 15% that currently have a pH this low. Both diatom-based and chrysophyte-based estimates of preindustrial to present-day changes in lake water chemistry show that approximately 25–35% of the target population has acidified. The extent and magnitude of acidification were greatest in the low-alkalinity lakes, for which we estimate that ~80% of the target population lakes with present-day measured pH ≤ 5.2 and 30–45% of lakes with pH between 5.2 and 6.0 have undergone declines in pH and ANC and concomitant increases in $[Al_m]$. The acidified lakes are primarily located in the southwestern Adirondacks, an area with little geological ability to neutralize acidic deposition and receives the highest annual average rainfall in the region. The diatom-based estimates suggest that some of the higher alkalinity lakes (pH >6.5) have increased in pH and ANC, although this trend is not supported by the chrysophyte-based estimates. The chrysophyte-based estimates show a greater extent and magnitude of acidification when compared with the diatoms. The scaled chrysophytes are exclusively planktonic, are more abundant in the spring, and are therefore more likely to be responsive to pH decreases and $[Al_m]$ increases associated with snowmelt. Historical changes in [DOC] were small and show no consistent pattern in the acidified lakes. The magnitude of acidification estimated in this study is somewhat less than that of computer-based model estimates and suggests that neutralization of acidic inputs has been more important than previously estimated, and/or preindustrial lake water conditions were more acidic than was originally assumed. These estimates of historical changes in lake water chemistry represent the first statistically based regional evaluation of the extent of lake acidification in the Adirondack region, as well as the only paleolimnological study ever conducted that was extrapolated to a statistically defined population of lakes.

Acknowledgements

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FIG. 8. Distributions of (A) pre-1850 pH inferred from diatoms, (B) present-day to pre-1850 change in pH as estimated from diatom inferences, (C) pre-1850 pH inferred from chrysophytes, and (D) present-day to pre-1850 change in pH as estimated from chrysophyte inferences.

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Allozyme Genotype and Differential Resistance to Mercury Pollution in the Caddisfly, *Nectopsyche albida*. I. Single-Locus Genotypes

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Benton, M. J., and S. I. Guttman. 1992. Allozyme genotype and differential resistance to mercury pollution in the caddisfly, *Nectopsyche albida*. I. Single-locus genotypes. Can. J. Fish. Aquat. Sci. 49: 142–146.

The caddisfly, *Nectopsyche albida*, was exposed to 0.6 mg mercury nitrate/L for 72 h and individual times to death recorded. All individuals were analyzed electrophoretically at six polymorphic loci. Time to death differed significantly among genotypes at the phosphoglucomutase and two alcohol dehydrogenase loci and among levels of heterozygosity. There is substantial evidence suggesting that many taxa possess allozyme genotypes exhibiting differential sensitivity to anthropogenic contaminants and natural stressors. Elimination of sensitive genotypes may reduce the ability of impacted populations to recover from additional impacts and may lessen the likelihood that they will adapt to slowly changing environmental conditions. Accordingly, we emphasize that (1) genetic standardization of bioassay organisms is necessary to assure consistency of results among testing facilities, (2) genetic monitoring may be a more sensitive method than numerical censusing to detect low levels of anthropogenic pollution, and (3) loss of genetic variation may hinder population persistence.

Des phryganes, *Nectopsyche albida*, ont été exposées à une concentration de nitrate de mercure de 0,6 mg/L pendant 72 h et les temps de survie individuels ont été notés. Tous les individus ont fait l'objet d'une analyse électrophorétique portant sur six locus polymorphes. Les temps de survie de divers génotypes variaient de façon significative pour le locus de la phosphoglucomutase et deux locus de la déshydrogénase de l'alcool de même qu'en fonction du niveau d'hétérozygotie. Il y a de forts indices qu'un grand nombre de taxons possèdent des génotypes allosymes présentant une sensibilité différentielle aux contaminants anthropiques et aux facteurs de stress naturels. La disparition des génotypes sensibles pourrait avoir pour effet de réduire la capacité des populations touchées à se remettre d'autres effets et à s'adapter aux conditions environnementales évoluant lentement. Par conséquent, nous soulignons que 1) la standardisation génétique des organismes servant aux essais biologiques s'avère nécessaire à l'uniformité des résultats entre laboratoires, 2) le contrôle génétique pourrait s'avérer plus sensible que le dénombrement comme méthode de détection de faibles niveaux de pollution anthropique et 3) la perte de variation génétique peut nuire au maintien de la population.

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Certain electrophoretically defined genotypes have been associated with differential tolerance to the effects of environmental stressors, or have been found in higher frequencies than other genotypes in impacted areas. This relationship has been shown for marine shrimp (Ben-Shlomo and Nevo 1988), marine and freshwater molluscs (Lavie and Nevo 1982, 1986a, 1986b; Lavie et al. 1984; Johannesson et al. 1990; Benton et al., in prep.), freshwater mayflies (Benton and Guttman 1990), and several fish species (Mitton and Koehn 1975; Smith et al. 1983; Gillespie and Guttman 1989; Chagnon and Guttman 1989a, 1989b; Diamond et al. 1989; Newman et al. 1989; Benton et al., in prep.). Heterozygosity level has also been associated with differential resistance (Diamond et al. 1989; Newman et al. 1989) and with other fitness-related traits such as size, growth rate, metabolic efficiency, and fecundity (Singh and Zouros 1978; Zouros et al. 1980, 1983, 1988; Koehn and Gaffney 1984; Koehn et al. 1988).

This growing body of evidence indicates that anthropogenic pollutants may eliminate or decrease the frequency of sensitive genotypes in affected populations. Such loss of variation may reduce the likelihood that a population will recover from additional episodes of stress, or may reduce its ability to adapt to slowly changing conditions.

While fish and marine invertebrates have commonly been used in these studies, freshwater invertebrates remain largely ignored. Benthic invertebrates, particularly those occupying lotic systems, are especially suited for this type of investigation because their habitat is frequently the depository for agricultural and industrial wastes and because their position in lotic food webs makes the preservation of genetically adaptable populations essential to the success of the entire lotic community.

We tested the relative sensitivity of allozyme variants and heterozygosity levels of the leptocerid caddisfly, *Nectopsyche albida* (Walker), which is widely distributed, easily collected, and not commonly used in toxicological or ecological genetic studies. Rather than the often-used comparison of genotype frequencies before and after some arbitrarily selected (and possibly biologically irrelevant) exposure time, we employed statistical methods that enabled the inclusion of both survivorship and failure-time data to test the relationship of each genotype with survival time over the entire exposure period.

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Methods

Nectopsyche albida (Leptoceridae:Trichoptera) larvae were collected from Lakengren Lake in Butler County, Ohio, returned to the laboratory, and placed in aerated reconstituted soft water (pH = 7.2–7.6; hardness = 40–48 mg $CaCO_3$/L; alkalinity = 30–35 mg $CaCO_3$/L) (USEPA 1975). Caddisflies were acclimated to laboratory conditions (20°C; 12 h light : 12 h dark) for at least 7 d and then transferred to 2-L crystallizing dishes containing 1.0 L of mercury nitrate solution. We used a mercury concentration previously determined to produce approximately 80% mortality over 72 h (0.6 mg Hg/L), with reconstituted soft water as the diluent, and a 14.5-cm-square piece of 1-mm-mesh nylon screen placed on the dish bottom to provide a grasping surface.

Ten experimental and two control replicates of 50 caddisflies per replicate were used. Controls were used only as a check against nontreatment-induced mortality and were not examined electrophoretically. The mercury solution was replaced every 24 h, and containers were checked for mortalities every 6 h around the clock. Previous work had shown that most enzymes were still active and electrophoretically scorable after 24 h at 20°C. A caddisfly was considered dead when it could no longer maintain its grip on the nylon screen, or when it failed to retract its legs into its case in response to prodding with forceps. Dead larvae were removed, frozen at −70°C, and the day and time of death were recorded. At the conclusion of the experiment, survivors were also frozen.

For electrophoresis, individual caddisflies were manually ground in an equal volume of 0.25 M surcrose in 2% (v/v) 2-phenoxyethanol solution. Horizontal starch gel (13–15% Sigma starch, Sigma Chemical Co., St. Louis, MO) electrophoretic techniques were employed for the resolution of genetic loci. Amine citrate pH 6.1 gels (Clayton and Tretiak 1972) were used for the resolution of phosphoglucomutase (PGM), glucosephosphate isomerase (GPI), triosephosphate isomerase (TPI), isocitrate dehydrogenase (IDH), and hexokinase (HEX-1). Phosphate pH 7.0 gels (Sweeney et al. 1987) were used for the resolution of malic enzyme (ME), alcohol dehydrogenase (ADH-1, ADH-2), esterases (EST), and hexokinase (HEX-2). These 10 enzyme loci were found to be polymorphic in a preliminary electrophoretic survey of *N. albida*. Banding patterns were interpreted by classifying the fastest migrating isozyme of an enzyme system as "1," the next fastest as "2," and so on, and by classifying the fastest migrating allozyme at a particular locus as "A," the next fastest as "B," and so on.

Data were analyzed by first using BIOSYS-1 (Swofford and Selander 1981), to test genotypic frequencies and heterozygosity for conformity to a Hardy–Weinberg distribution. The effects of replicate, genotype, and heterozygosity level on time to death (TTD) were tested using the SAS LIFEREG procedure (SAS Institute Inc. 1985), which fits parametric models to failure-time and right-censored (i.e. survivorship) data (see also Diamond et al. 1989). This program tests the effect of each independent variable (allozyme genotype, heterozygosity level) on the dependent variable (TTD) while controlling for the remaining independent variables (see footnotes to Table 1). Overdilution of some tissues and other technical problems prevented the resolution of genotypes at all loci for every individual and resulted in unequal sample sizes (see Table 1). However, the LIFEREG procedure accommodates unequal sample sizes (SAS Institute Inc. 1985).

Results

Of the 446 treated caddisflies recovered over the course of the experiment, 383 (86%) were dead at the end of 72 h and 63 (14%) survived. The remaining 54 of the original 500 treated animals (11%) are believed to have been cannibalized, as only their empty cases were found. No emerged adults were found, and their escape was prevented by plastic film covering each dish. No mortalities were observed in the control replicates, although a similar percentage (8%) was lost, apparently to cannibalism.

Electrophoretic analysis revealed five alleles and 10 genotypes for PGM, three alleles and four genotypes for GPI, four alleles and five genotypes for TPI, two alleles and two genotypes for ME, and three alleles and six genotypes each for ADH-1 and ADH-2. EST, HEX, and IDH were not consistently scorable for experimental animals, even though bands for control animals on the same gel were quite clear. This was possibly due to interference by mercury on enzyme activity.

A deficiency of heterozygotes existed for ADH-2 (chi-square = 16.15, $p < 0.01$). This appeared to be related to 11 fewer than expected AB genotypes and a concomitant 11 more than expected AA genotypes. Because the frequency of the AA genotype was relatively low (0.06), genotypes were pooled and this eliminated the heterozygote deficiency (chi-square = 1.75, $p > 0.10$).

There were no significant differences in TTD among replicate treatments (chi-square = 1.06, $p > 0.40$), so data from all treatments were pooled. TTD differed significantly among PGM, ADH-1, and ADH-2 genotypes and among levels of heterozygosity (see Tables 1 to 5). There were no significant differences in TTD among PGI (chi-square = 3.02, $p > 0.30$), TPI (chi-square = 8.12, $p > 0.05$), or ME (chi-square = 1.19, $p > 0.20$) genotypes (but see Benton and Guttman 1992 for multilocus analyses).

Increasing heterozygosity over a range of zero to four loci was associated with a trend of increasing TTD (Table 1). Median TTD for individuals heterozygous at a single locus was significantly higher than TTD for individuals homozygous at all tested loci, and three- and four-locus heterozygosity was associated with a TTD significantly higher than that of two-locus heterozygotes (Tables 1 and 5). Because heterozygosity at five and six loci occurred in only single individuals, it was not possible to statistically compare them.

Discussion

Allozyme electrophoresis is providing mounting evidence for the existence of "preadapted" genotypes that are relatively resistant to the toxic effects of anthropogenic contaminants. In this study, genotypes at the PGM, ADH-1, and ADH-2 loci were associated with a wide range of failure times (Tables 1 and 2).

PGM has been previously associated with differential responses to heavy-metal toxicity in fishes (Gillespie and Guttman 1989) and shrimp (Ben-Shlomo and Nevo 1988). Other allozymes have also shown a relationship between genotype and heavy-metal sensitivity in aquatic animals, including snails (Lavie and Nevo 1982, 1986a, 1986b), fishes (Chagnon and Guttman 1989a; Diamond et al. 1989; Newman et al. 1989), and mayflies (Benton and Guttman 1990).

This laboratory evidence is strengthened by field studies that also suggest that certain PGM genotypes are selected for under

TABLE 1. Median times to death (h) for *N. albida* allozyme genotypes and heterozygosity levels. Standard errors are in parentheses. Footnotes give genotypes and heterozygosity level held constant during analysis of each locus.

PGM[a]			ADH-1[b]			ADH-2[c]			Heterozygous loci[d]		
	TTD	*N*		TTD	*N*		TTD	*N*	Level	TTD	*N*
AA	58.1 (1.4)	2	AA	109.7 (2.2)	13	AA	29.0 (2.1)	23	0	48.8 (2.0)	35
AC	50.6 (1.8)	17	AB	77.5 (2.2)	71	AB	42.8 (2.1)	64	1	60.1 (1.9)	69
AD	54.0 (1.8)	30	AC	58.8 (1.8)	23	AC	57.2 (1.8)	21	2	52.0 (1.9)	64
BC	30.5 (1.9)	3	BB	62.2 (2.3)	140	BB	55.1 (2.3)	148	3	62.0 (1.9)	75
BD	55.6 (1.8)	12	BC	25.7 (1.6)	84	BC	21.4 (1.4)	92	4	64.2 (1.9)	20
CC	71.8 (1.8)	45	CC	58.1 (1.4)	9	CC	58.1 (1.4)	11	5	50.5 (2.2)	1
CD	58.0 (1.7)	157							6	58.1 (1.4)	1
CE	35.4 (2.1)	1									
DD	65.7 (1.7)	173									
DE	30.1 (2.0)	2									

[a]Constants: ADH-1-CC, ADH-2-CC, heterozygosity level 6.
[b]Constants: PGM-AA, ADH-2-CC, heterozygosity level 6.
[c]Constants: PGM-AA, ADH-1-CC, heterozygosity level 6.
[d]Constants: PGM-AA, ADH-1-CC, ADH-2-CC.

TABLE 2. Pairwise comparisons of median times to death (h) for *N. albida* PGM genotypes. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	AA	AC	AD	BC[a]	BD	CC	CD	CE	DD	DE
AA	—	NS	NS	*	NS	NS	NS	NS	NS	NS
AC		—	NS	*	NS	*	NS	NS	*	NS
AD			—	*	NS	*	NS	NS	*	NS
BC[a]				—	*	***	**	NS	NS	NS
BD					—	NS	NS	NS	NS	NS
CC						—	*	*	NS	**
CD							—	NS	*	*
CE								—	NS	NS
DD									—	*
DE										—

[a]$N = 3$.

TABLE 3. Pairwise comparisons of median times to death (h) for *N. albida* ADH-1 genotypes. *$p < 0.05$, *** $p < 0.001$.

	AA	AB	AC	BB	BC	CC
AA	—	*	NS	NS	***	NS
AB		—	NS	NS	***	NS
AC			—	NS	***	NS
BB				—	***	NS
BC					—	***
CC						NS

TABLE 4. Pairwise comparisons of median times to death (h) for *N. albida* ADH-2 genotypes. * $p < 0.05$, *** $p < 0.001$.

	AA	AB	AC	BB	BC	CC
AA	—	*	*	*	***	NS
AB		—	NS	NS	***	NS
AC			—	NS	***	NS
BB				—	***	NS
BC					—	***
CC						—

TABLE 5. Pairwise comparisons of median times to death (h) for *N. albida* heterozygosity levels (0 to 6 loci). * $p < 0.05$.

	0	1	2	3	4	5	6
0	—	*	NS	NS	NS	NS	NS
1		—	NS	NS	NS	NS	NS
2			—	*	*	NS	NS
3				—	NS	NS	NS
4					—	NS	NS
5						—	NS
6							—

conditions of anthropogenic contamination and other environmental stressors. PGM genotypes of the stoneroller, *Campostoma anomalum*, shown to be copper sensitive in laboratory studies, occurred in lower frequencies in populations occupying radionuclide-impacted sites than in populations upstream from the impact (Gillespie and Guttman 1989). Selection at the PGM (Thiesen 1978) and other loci (Koehn 1978; Koehn et al. 1980, 1984; Hilbish et al. 1982; Hilbish and Koehn 1985; Bulnheim and Gosling 1988) under varying conditions of temperature and salinity has been well documented in the blue mussel, *Mytilus edulis*. Both the snail, *Helisoma trivolvis*, and the mosquitofish, *Gambusia affinis*, show profound differences in allele and genotype frequencies between populations from adjacent clean and polluted ponds (Benton et al., in prep.), and allelic frequencies of isozymes in the mummichog, *Fundulus heteroclitus*, and the largemouth bass, *Micropterus salmoides*, differed significantly between populations from artificially heated and unheated waters (Mitton and Koehn 1975; Smith et al. 1983).

ADH has not previously been associated with genotype-specific response to heavy metals, but ADH genotype frequencies do respond significantly in mussels to shifts in salinity (Johannesson et al. 1990) and in *Drosophila* to general environment type (Munoz-Serrano et al. 1985).

Resistance to mercury in *N. albida* also appears to increase as heterozygosity level increases over a range of zero to four loci (Table 1). Heterozygosity level has previously been correlated with resistance to heavy-metal exposure in mosquitofish (Diamond et al. 1989; Newman et al. 1989). This analysis of multilocus heterozygosity is in contrast with our intralocus comparisons of heterozygotes and homozygotes. Both ADH-1 and ADH-2 homozygotes were associated with the longest and third longest TTD's, while PGM homozygotes were associated with the three longest TTD's. Others have also found homozygous allozyme genotypes to be selectively favored under toxic conditions (Lavie and Nevo 1986b; Diamond et al. 1989). So,

while differential resistance may be associated with homozygous genotypes at individual loci in some cases, the positive effect of heterozygosity per se appears to be an additive one (see also Diamond et al. 1989; Newman et al. 1989).

The mechanisms that cause specific allozyme genotypes to fail sooner than others are not yet fully understood. There is in vitro evidence that the activity of some enzymes is reduced or eliminated by the competitive inhibition of heavy-metal ions (Milstein 1961; Mizrahi and Achituv 1989). Chagnon and Guttman (1989b) found that two PGM genotypes differed in staining intensity when copper sulfate was added to the electrophoretic stain mixture. In the current study, electrophoretic staining of esterases was inhibited in caddisflies that were exposed to mercury, but not inhibited in untreated caddisflies unless mercury was added to the stain mixture (Benton and Guttman, in review). Increasing concentrations of mercury added to esterase stain mixtures resulted in decreasing resolution of esterase banding patterns, with maximal inhibition at approximately the same mercury concentration at which caddisflies were tested. Heavy metals are also known to interfere with chloride cell oxidative phosphorylation (Crespo and Sala 1986) and gill tissue respiration (Brown and Newell 1972), although their direct affect on enzyme function in these cases is not clear. It should be noted, however, that electrophoretically detectable enzymes may merely be linked to other loci that produce observed differential effects (Diamond et al. 1989), or may act as markers for much of, or entire, chromosomes that are differentially affected (Beaumont et al. 1989).

As evidence for genotype-specific responses to stress increases, the important practical implications become clear. First, genetic differences in bioassay organism cultures may produce contradictory results. Baird et al. (1989) found a 32-fold difference in cadmium chloride 48-h LC_{50}'s for various clones of *Daphnia magna*. Larval *Chironomus tentans* differed significantly in heterozygosity level among seven U.S. laboratory populations (Woods et al. 1989). Standard bioassay organisms such as these should be genetically standardized within and among testing sites. This is particularly important for facilities upon whose results water quality and other environmental guidelines are based. Parthenogenetic species such as *Daphnia* should be maintained in pure clonal lines that reflect those occurring in relevant natural populations and should be periodically tested for purity due to the possibility of mutation (Baird et al. 1989). Conversely, sexually reproducing species should be outbred regularly to avoid establishment of genetically uniform lines and to ensure that allele and genotype frequencies and mean heterozygosity levels approximate those found in natural populations. These cultures also should be regularly tested to monitor genetic diversity.

Second, genetic monitoring of populations may be a much more sensitive method than numerical censusing for detecting sublethal levels of contamination (see Ben-Shlomo and Nevo 1988). In ecologically sensitive habitats, organisms from several trophic levels might be genetically tracked by electrophoresis so that seasonal, generational, and stochastic shifts in genetic patterns can be documented. A sudden shift in allele or genotype frequency should warrant investigation of habitat quality.

Finally, because resistant individuals may represent as little as 0.02% of a population (Bradshaw and Hardwick 1989), a great deal of genetic variation may be lost from an impacted population through toxic contamination. The yellow perch, *Perca flavescens*, of Lake Erie, for example, thought to have recovered from overfishing and pollution-related decimation, now have virtually no detectable variation (Strittholt et al. 1988). Such wholesale loss of sensitive alleles from impacted populations may seriously reduce the likelihood that those populations will recover from further episodes of impact or from natural catastrophes. Indeed, genotypes resistant to one stressor may actual be sensitive to others (Ben-Shlomo and Nevo 1988). More importantly perhaps, loss of gentic variation may impede adaptation to gradual, long-term changes in environmental conditions. Populations that do persist may experience decreases in fitness, lack of range expansion (Parsons 1990), or range reduction due to stress-related increases in metabolic rate (Koehn and Bayne 1989; Hoffmann and Parsons 1989). In light of the apparent adaptive significance of genetic polymorphism (Hedrick 1986; Nevo and Beiles 1988), this is particularly relevant for populations that inhabit highly variable, unpredictable, or stressful environments or occupy species boundaries (Parsons 1990).

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Allozyme Genotype and Differential Resistance to Mercury Pollution in the Caddisfly, *Nectopsyche albida*. II. Multilocus Genotypes

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Benton, M. J., and S. I. Guttman. 1992. Allozyme genotype and differential resistance to mercury pollution in the caddisfly, *Nectopsyche albida*. II. Multilocus genotypes. Can. J. Fish. Aquat. Sci. 49: 147–149.

While a number of papers document that sensitivity to pollution is correlated with single-locus genotype, only one has addressed associations with multilocus complexes. We exposed larval caddisflies, *Nectopsyche albida*, to inorganic mercury and recorded individual times to death, genetically characterized each individual at six polymorphic loci by starch gel electrophoresis, and tested the effects of multilocus genotype on time to death. Two two-locus complexes and two three-locus complexes were significantly correlated with survival time. This supports earlier studies that monitoring multilocus and single-locus genotype frequencies may be useful in detecting and measuring environmental impacts; however, we disagree that variation in survival time among genotypes per se supports selectionist theory, because no heritability of resistance has been demonstrated. We also disagree that enzyme systems not exhibiting such variation are nonadaptive and discuss how the elimination of sensitive multilocus genotypes may hinder population persistence.

Bon nombre de communications montrent que la sensibilité à la pollution est corrélée à un génotype à un seul locus, mais une seule traite des corrélations avec des complexes à locus multiples. Nous avons exposé des larves de phryganes, *Nectopsyche albida*, à du mercure inorganique et noté les temps de survie individuels, caractérisé chaque individu à six locus polymorphes par électrophorèse sur gel d'amidon et testé les effets du génotype à locus multiples sur le temps de survie. Deux complexes à deux locus et deux complexes à trois locus présentaient une corrélation significative avec le temps de survie. Cela appuie des études antérieures selon lesquelles le contrôle des fréquences des génotypes à plusieurs ou à un seul locus pourrait être utile pour la détection et la mesure des incidences sur l'environnement, mais nous ne croyons pas que la variation du temps de survie en fonction des génotypes appuie en elle-même la théorie basée sur la sélection car aucune héritabilité de la résistance n'a pu être démontrée. Nous réfutons aussi l'hypothèse selon laquelle les systèmes enzymatiques ne présentant pas une telle variation ne soient pas adaptatifs et nous traitons de la façon dont la disparition des génotypes à locus multiples sensibles peut nuire à la persistance de la population.

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Recent research has shown that, in both vertebrate and invertebrate taxa, certain electrophoretically defined single-locus allozyme genotypes are associated with differential resistance to various stressors, or occur in higher frequencies in polluted habitats. Multilocus heterozygosity (i.e. number of heterozygous loci) has also been correlated with differential resistance (see Benton and Guttman 1992 for review).

Only a single study (Lavie and Nevo 1988), however, has dealt with the relationship of multilocus allozyme genotypes to pollution resistance, and those authors tested only multilocus complexes that excluded enzyme systems exhibiting differential survivorship at the single-locus level (reported in Lavie and Nevo 1986). Genotype frequencies apparently were compared between live and dead fractions from several heavy-metal bioassays, which were run until LD_{50}'s were achieved, but the exact methodology is unclear. Two four-locus complexes and a single two-locus complex were found to exhibit resistance to two heavy metals. The authors contended that their results were "concordant with the selectionist theory of the genetic variability" and that single enzyme systems not exhibiting differentially tolerant genotypes were "nonadaptive."

In the current study, we tested the effects of all two- and three-locus enzyme combinations on the median time to death (TTD) of the leptocerid caddisfly, *Nectopsyche albida* (Walker), under conditions of inorganic mercury pollution. We compare our results with those of Lavie and Nevo (1988), and we take issue with their interpretation. We also address the ecological and evolutionary implications of differential sensitivity to pollutants among multilocus genotypes.

Materials and Methods

Experimental and statistical methods were the same as in Benton and Guttman (1992). Multilocus complexes were computer-generated from all possible two- and three-locus genotype combinations of the six enzyme systems tested, using only those genotypes that occurred 10 or more times.

Results

Two-Locus Complexes

Significant effects on TTD were found among genotypes of two two-locus complexes: TPI/ADH-1 (chi-square = 9.63,

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TABLE 1. Median times to death (h) for two- and three-locus genotype complexes in *N. albida*. Standard errors are in parentheses.

TPI/ADH-1	ADH-1/ADH-2	TPI/ME/ADH-2	TPI/ADH-1/ADH-2
AAAB 55.5 (1.10)	AAAA 52.3 (1.11)	AAAAAA 43.9 (1.10)	AAABAB 57.4 (1.05)
AAAC 56.4 (1.14)	ABAA 61.0 (1.30)	AAAAAB 57.7 (1.23)	AAACAC 59.0 (1.17)
AABB 59.0 (1.03)	ABAB 59.0 (1.26)	AAAAAC 60.9 (1.28)	AABBBB 57.8 (1.12)
AABC 48.5 (1.10)	ACAC 60.3 (1.28)	AAAABB 58.4 (1.22)	AABCBC 48.6 (1.12)
ABBB 52.8 (1.15)	BBBB 58.7 (1.25)	AAAABC 51.6 (1.22)	ABBBBB 52.9 (1.17)
	BCBC 58.8 (1.25)	ABAABB 57.6 (1.28)	

TABLE 2. Pairwise comparisons of median times to death (h) between TPI/ADH-1 genotype complexes. ** $p < 0.01$.

	AAAB	AAAC	AABB	AABC	ABBB
AAAB	—	NS	NS	NS	NS
AAAC		—	NS	NS	NS
AABB			—	**	NS
AABC				—	NS
ABBB					—

TABLE 3. Pairwise comparisons of median times to death (h) between ADH-1/ADH-2 genotype complexes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	AAAA	ABAA	ABAB	ACAC	BBBB	BCBC
AAAA	—	*	NS	NS	NS	NS
ABAA		—	***	**	**	*
ABAB			—	NS	NS	*
ACAC				—	NS	*
BBBB					—	*
BCBC						—

$p < 0.05$) and ADH-1/ADH-2 (chi-square = 17.58, $p < 0.01$). Among TPI/ADH-1 genotypes, TTD was significantly longer for individuals with the AA/BB genotype than for individuals of the AA/BC genotype (chi-square = 9.02, $p < 0.01$) (Tables 1 and 2). Among ADH-1/ADH-2 genotypes, TTD differed significantly among a number of combinations (chi-squares = 3.77–10.87, $p < 0.001$–0.05) (see Tables 1 and 3).

Three-Locus Complexes

Significant effects on TTD were found among genotypes of two three-locus complexes: TPI/ME/ADH-2 (chi-square = 10.84, $p < 0.05$) and TPI-ADH-1/ADH-2 (chi-square = 10.71, $p < 0.05$). Among TPI/ME/ADH-2 genotypes, TTD was significantly shorter for individuals with the AA/AA/AA genotype than for those with the AA/AA/AB, AA/AA/AC, or AA/AA/BB genotype (chi-squares = 2.16–7.17, $p < 0.05$–0.01). In addition, individuals of the AA/AA/BB genotype survived a significantly longer time than did individuals of the AA/AA/BC genotype (chi-square = 3.98, $p < 0.05$) (Tables 1 and 4). Among TPI/ADH-1/ADH-2 genotypes, individuals with the AA/BC/BC genotype exhibited a significantly shorter TTD than individuals of either the AA/AB/AB or AA/BB/BB genotype (chi-squares = 5.88 and 8.68, respectively, $p < 0.05$ and 0.01, respectively) (Tables 1 and 5).

Discussion

Our results demonstrate that susceptibility to mercury pollution in *N. albida* varies not only among single-locus allozyme genotypes and levels of heterozygosity (Benton and Guttman 1992), but also among multilocus complexes.

We agree with Lavie and Nevo (1988) that monitoring multilocus complexes as well as single-locus allozyme genotype frequencies in organisms occupying ecologically sensitive habitats may be useful as an indicator of pollution. At both levels, shifts in allele or genotype frequencies in favor of known resistant genotypes may serve as an early indicator of environmental impact. These methods may not only be more sensitive than numerical censusing, but they may obviate problems of sampling interval, spatial scaling, and time lags inherent in census-type monitoring programs (Underwood 1989).

We disagree with Lavie and Nevo (1988), however, that these kinds of results per se support the selectionist model of genetic variability and that single-locus systems not exhibiting differentially surviving genotypes are individually "nonadaptive." These arguments are, in fact, contradictory.

First, when genotypes vary in resistance to stress, genetic changes in the population may occur in the direction of increased resistance (Bradshaw and Hardwick 1989). However, while differential resistance to pollutants in a newly impacted population indicates that fortuitous "preadapted" genotypes or "hidden variation" (Cooper 1954) exist (Bradshaw and Hardwick 1989), it is not an indication that such resistance is necessarily heritable (Lawrence 1984) because continued application of the stressor can lead to death, even in individuals that are able to initially react with some compensatory (but energetically expensive) response (Gray 1989; Sibly and Calow 1989).

TABLE 4. Pairwise comparisons of median times to death (h) between TPI/ME/ADH-2 genotype complexes. * $p < 0.05$, ** $p < 0.01$.

	AAAAAA	AAAAAB	AAAAAC	AAAABB	AAAABC	ABAABB
AAAAAA	—	*	*	**	NS	NS
AAAAAB		—	NS	NS	NS	NS
AAAAAC			—	NS	NS	NS
AAAABB				—	*	NS
AAAABC					—	NS
ABAABB						—

TABLE 5. Pairwise comparisons of median times to death (h) between TPI/ADH-1/ADH-2 genotype complexes. * $p < 0.05$, ** $p < 0.01$.

	AAABAB	AAACAC	AABBBB	AABCBC	ABBBBB
AAABAB	—	NS	NS	*	NS
AAACAC		—	NS	NS	NS
AABBBB			—	**	NS
AABCBC				—	NS
ABBBBB					—

Second, when differential resistance is not found in a given enzyme system, it is not necessarily because it does not occur. Differential responses may be distinguishable only at other life stages of the organism, at a higher or lower toxicant concentration, or over a shorter or longer exposure duration. Differential responses might also be measurable in traits other than survivorship (see Koehn and Bayne 1989). In any case, the selectionist model dictates that genotypes occurring in high frequencies within a large population are almost certainly the result of adaptation through natural selection, lack of differential tolerance to a selected anthropogenic stressor notwithstanding.

Despite these points of disagreement, we concur that the potential for major genetic shifts through the selective elimination of sensitive genotypes and the proliferation of resistant ones is high. The risks inherent in such reduced genetic variability (see Benton and Guttman 1992) may increase when genotype complexes evolve in concert. Because selection can act on multilocus combinations (Franklin and Lewontin 1970), individuals with all but specific resistant suites of genotypes may be eliminated by anthropogenic contamination. If we assume independent segregation of alleles, these individuals will represent a decreasing proportion of the population as the coadapted complex of genotypes increases in size. Consequently, an increasing number of individuals will be lost with initial exposure, and the likelihood of population persistance is lessened.

It is becoming increasingly evident that pollutants threaten the genetic variability that underlies the success of natural populations (Westman and Kallio 1986; Stritholt et al. 1988; Rapport 1989). It is essential to maintain wild plant and animal populations with sufficient genetic plasticity to meet natural and man-made challenges. It seems reasonable to suggest that monitoring sensitive populations for genetic as well as phenotypic and demographic changes may provide early warning of events which precede shifts in population and community stability.

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A Multivariate Model for Predicting Population Fluctuations of *Dreissena polymorpha* in North American Lakes

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The European zebra mussel, *Dreissena polymorpha* (Pallas), has recently been introduced to North American freshwaters. *Dreissena* exhibits a wide range of population dynamics among different European lakes. In some lakes, long-term population size is fairly constant, while populations in other lakes can fluctuate greatly from year to year. Assessments of the potential ecological and economic effects of *Dreissena* in North America will be affected if populations show large interannual fluctuations in abundance. We develop a multivariate model for predicting population dynamics of *Dreissena* in novel lake systems. Our model uses the limnological differences between lakes with constant populations of *Dreissena* and those with variable populations to identify variables that may determine population dynamics. We find that lakes with more constant populations tend to have larger surface areas, lower levels of calcium, and higher levels of phosphate (PO_4) compared with lakes with more variable populations.

La dreissénie européenne, *Dreissena polymorpha* (Pallas), a récemment été introduite dans les eaux douces de l'Amérique du Nord. Les *Dreissena* des lacs européens présentent une large gamme de dynamiques de population. Dans certains lacs, la taille de la population est passablement constante à long terme tandis que les populations d'autres lacs fluctuent grandement d'une année à l'autre. Les évaluations des effets écologiques et économiques éventuels des *Dreissena* en Amérique du Nord donneront des résultats variables si les populations présentent d'importantes fluctuations annuelles d'abondance. Nous avons élaboré un modèle à variables multiples pour la prévision de la dynamique des populations des *Dreissena* dans de nouveaux lacs. Le modèle fait appel aux différences limnologiques entre les lacs à populations constantes de *Dreissena* et ceux où les populations fluctuent pour la détermination des variables pouvant influer sur la dynamique des populations. Nous avons trouvé que les lacs à populations plus constantes avaient tendance à présenter une plus grande surface, des teneurs en calcium moins importantes et des teneurs en phosphate (PO_4) plus élevées que les lacs à populations plus variables.

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Since the turn of the century exotic invaders have greatly influenced the ecological history of many North American lakes, especially the Great Lakes (Christie et al. 1972; Smith and Tibbles 1980). For example, the abundance, growth habit, and population dynamics of lamprey (*Petromyzon marinus*), smelt (*Osmerus mordax*), and alewife (*Alosa pseudoharengus*) have had wide-ranging ecological effects. The most recent major invader, the European zebra mussel, *Dreissena polymorpha* (Pallas), has characteristics of other successful invaders (Hengeveld 1989). It has a high reproductive rate and a growth habit and feeding type that were not previously represented in these newly colonized lakes. In the past we have had to learn about the ecological effects of aquatic invaders over many years as communities responded to invasion and to the subsequent population dynamics of the new species. However, enough is known about *Dreissena*'s physiology, life history, and ecology to develop predictive models of its ecological effects based on analyses of published data. We are using this approach in this paper to predict how environmental factors may affect the population dynamics of *Dreissena* in North American lakes.

Populations of *Dreissena* are now found in all Great Lakes basins (New York Zebra Mussel Information Clearinghouse 1990; Griffiths et al. 1991). They were introduced in the mid-1980's, probably through ballast water transport from ships (Hebert et al. 1989). The zebra mussel has also extended its range through most of Europe over the last two centuries (Wesenbrug-Lund 1939; Stańczykowska 1977). The European experience forewarns of ecological and economic effects on North American freshwater ecosystems caused by *Dreissena* biofouling and feeding (Mackie et al. 1989).

Fluctuations in the size of zebra mussel populations can cause ecological consequences throughout a community (Carpenter 1988; Mills and Forney 1988). The life history of top predators can impose a periodicity which affects other system components (Carpenter and Leavitt 1991). Invasion of *Dreissena* may produce similar effects. With their high feeding rates, shoals of these benthic filter feeders can remove large percentages of primary productivity, which may reduce the energy available to pelagic food webs (Stańczykowska 1977; Mackie et al. 1989; Schneider 1991). Fluctuations in populations of *Dreissena*, whether caused by extrinsic factors or density dependence, may affect dynamics of phytoplankton, and thereby indirectly affect herbivorous zooplankton competitors. Fish recruitment and growth may also be affected by *Dreissena* population fluctua-

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tions. Although the effects of *Dreissena* on fish populations have not been thoroughly studied, dense overgrowths of zebra mussels on rocky fish spawning beds may deplete oxygen, causing egg mortality (O'Neill and MacNeill 1989). Small zebra mussels are consumed by many species of fish (Stańczykowska 1977). Interannual fluctuations in *Dreissena* abundance may affect the zooplankton and mussel forage base available to fish, and perhaps fish recruitment. *Dreissena* population dynamics may also be economically important. Biofouling of intake pipes, spawning beds, ships' hulls, and other waterworks may be severe enough to require management responses only during years of high abundance.

Reports of *Dreissena* population fluctuations seem to fall into two categories: (1) invasion dynamics and (2) irregular population increases and decreases (Stańczykowska 1977). A third pattern may be a regular cycle of population abundance, although only one study has demonstrated a weak periodicity (Lewandowski 1982). In Lake Mikołajskie, Poland, two population peaks appear 16 yr apart during a 20-yr period (Lewandowski 1982). A common invasion dynamic is an initial high abundance followed by a crash to low levels (Schlesch 1930; Sebestyén 1938). The boom-and-bust invasion of Lake Constance, Germany, is a well known example of this pattern (Walz 1974). The type of dynamic most often observed is an irregular pattern of population increases and decreases (Stańczykowska et al. 1975b) (Fig. 1).

The degree to which populations irregularly fluctuate varies widely among European lakes (Kajak and Dusoge 1975; Stańczykowska et al. 1975b). In some lakes, *Dreissena* abundance varies by no more than 15% among years over periods of 5–10 yr. In other lakes, populations can decline by two orders of magnitude within a few months and recover to their original level in several years (Stańczykowska et al. 1975b).

Several different mechanisms may underlie these population fluctuations, including resource depletion (e.g. food, oxygen, or attachment surfaces; Stańczykowska 1977), occasional high abundance of predators and parasites, fluctuations in larval recruitment (Lewandowski 1982; MacIsaac et al. 1991), and environmental changes. Dense populations of *Dreissena* show signs of physiological stress (reduced body mass; Stańczykowska 1964). A combination of overcrowding and low midwinter oxygen levels have caused large die-offs of *Dreissena* (along with fish kills) in Lake Mikołajskie (Stańczykowska 1961; Stańczykowska et al. 1975b).

To allow better forecasts of the potential ecological and economic effects of *Dreissena* in North America, we develop a model for predicting *Dreissena* population fluctuations. We (1) quantify the potential range for interannual variance in population abundance, (2) identify limnological factors that may affect population variability, and (3) construct a model by using empirical, multivariate analysis of published data on the limnological environments of European lakes where different *Dreissena* populations have different dynamics. This is a powerful technique for predicting the behavior of a species during range expansion, based on its known population performance elsewhere (Pimm and Bartell 1980). This technique has already been successfully used to determine limnological factors that affect the distribution and abundance of native North American molluscs (Green 1971, 1972, 1974).

Methods

Published data on both *Dreissena* abundance and on the biotic and abiotic environments of the European lakes they inhabit were collected from a search of over 200 papers. These papers were found by searching through various American and European journals from 1900 to 1990, performing computerized library searches for key words (e.g. *Dreissena*) and authors (e.g. Stańczykowska, Lewandowski), and using the reference lists published in other papers on *Dreissena*. Of the more than 250 lakes for which data were collected, only 18 lakes had time-series data extending over a minimum of 10 yr and limnological data that were collected over a time period coincident to the *Dreissena* data for each lake (Appendix A).

Two of the lakes used in our analyses were in Germany (Lakes Stechlin and Nehmitz), one was in the USSR (Kuibyshev Reservoir), and the rest were in Poland's northern lake districts (Table 1). They were all studied between 1935 and 1975. *Dreissena* has occurred throughout these areas since the mid-1800's (Stańczykowska 1977). The lakes were in well-populated areas, and many received effluents from agriculture, industry, and municipal sewage (Kajak and Zdanowski 1983). Two lakes were oligotrophic, five were mesotrophic, and the rest were eutrophic. Water flow was controlled by dams in most of these lakes.

We made no a priori decisions about which lakes or variables should be retained in the dataset, but included data that would form as large a rectangular matrix as possible. The final dataset included 18 lakes and 17 limnological variables (Tables 1, 2). Several of the limnological variables that we used would have natural temporal variation on time scales of hours to years and spatial variation in both horizontal and vertical dimensions. For example, pH can show diurnal variations related to algal activity and can vary with water depth (Wetzel 1983). We dealt with this variation by averaging as many values as we could find for each parameter. Drainage basin area, lake surface area, and lake volume spanned four orders of magnitude within our dataset; therefore, values for these variables were log-transformed ($\log_{10}$) to prevent undue influences of large lakes in our mathematical analyses. All variables were approximately normally distributed (Kolmogorov–Smirnoff test of normality, $p \geq 0.04$, $\alpha = 0.003$ after adjustment by Bonferroni's inequalities for multiple comparisons; Snedecor and Cochran 1980).

The goal of our multivariate analyses was to distinguish lakes with variable populations of *Dreissena* from those with constant populations. We first categorized our lakes as having variable or constant populations based either on analysis of year-to-year fluctuations in *Dreissena* abundance or on studies of long-term population dynamics conducted by Stańczykowska et al. (1975b). We calculated coefficients of variation (V = (standard deviation/mean) × 100, and is expressed as a percentage; Sokal and Rohlf 1981) of *Dreissena* abundance for each lake in our dataset for which we could find at least three estimates of *Dreissena* abundance (average number of mussels per square metre in the zone of occurrence) within a 15-yr period. V standardizes variance against the magnitude of the mean, so that lakes with low variance and large populations of *Dreissena* would not appear to be more variable than those with low variance and small populations. The number of observations of *Dreissena* abundance varied from two to seven for each lake. Lakes with values of V under 50% were considered to have constant populations (four lakes), while those with $V > 50\%$ have variable populations (eight lakes). A threshold value of 50% divided the lakes into groups that agreed very well with reports of *Dreissena* population variability from authors who have long-term experience with these lake systems. Several lakes were classified as having either constant (three lakes) or

TABLE 1. Summary of *Dreissena* abundance data. "Density" is no. mussels·m^{-2} in the zone of occurrence averaged over several years (n). "Peak" is the peak density during that period, V is the coefficient of variation, and "criteria" is the method used to classify each lake as having either constant or variable populations (see text).

Country	Lake	Density	Peak	V	n	Criteria
			Lakes with constant populations			
Poland	Beldany	23.8	25.0	—	2	Stańczykowska et al. 1975b
Poland	Boczne	960.0	1400.0	10.8	3	V
Poland	Dargin	566.7	600.0	—	2	Stańczykowska et al. 1975b
Poland	Dobskie	566.7	600.0	—	2	Stanczykowska et al. 1975b
Poland	Niegocin	1060.0	1300.0	30.0	3	V
Poland	Sniardwy	510.0	600.0	17.8	3	V
Poland	Szymon	1500.0	1500.0	5.0	4	V
			Lakes with variable populations			
Poland	Goldapiwo	531.3	542.5	—	2	Stańczykowska et al. 1975b
Poland	Jagonde	570.0	1125.0	93.8	4	V
USSR	Kuibyshev	1370.9	6698.0	174.52	3	V
Poland	Mikołajskie	759.4	2200.0	116.6	7	V
Germany	Nehmitz	2574.0	2574.0	—	3	Other
Germany	Stechlin	7541.0	14435.0	81.7	3	V
Poland	Stregiel	1136.7	1700.0	71.4	3	V
Poland	Taltowisko	1850.0	4500.0	99.5	5	V
Poland	Talty	1222.5	3600.0	170.4	3	V
Poland	Wojnowo	562.5	1100.0	—	2	Stańczykowska et al. 1975b
Poland	Zabinki	1273.3	2000.0	70.5	3	V

variable (two lakes) populations based on the studies of Stańczykowska et al. (1975b). Estimates of *Dreissena* abundance in several lakes studied in 1962, and again in 1972, showed very large changes in population size in some lakes and nearly constant populations in others. Finally, one lake, Lake Nehmitz, was classified as having a variable population based on observations of *Dreissena* being abundant, rare, and then abundant again in succcessive years.

We used Discriminant Function Analysis (DFA) (SAS Institute Inc. 1990) on the limnological variables to find a mathematical function that best distinguishes lakes with constant from those with variable populations. We then used this model to predict *Dreissena* population dynamics in novel lake systems. For the DFA, each lake was assigned a prior probability of 1 for belonging to its own group (constant or variable) and 0 for belonging to the other group. A stepwise, parametric DFA identified which variables were most important in contrasting lakes with constant populations from those with fluctuating populations. Due to several missing values for variables, the stepwise DFA was conducted using all lakes except Beldany, Nehmitz, and Stechlin, and all variables except log(basin area), and chlorine. We ran a canonical DFA on the important variables identified by the stepwise DFA in order to create a predictive mathematical model. We tested the error rate of the model with a jacknife cross-validation technique.

Results

Dreissena has a wide range of potential population variability (Table 1; Fig. 1). Populations vary by only 10–30% over time scales of 5–10 yr in about half of the lakes in our dataset, while in the other half, populations can crash by over two orders of magnitude in a few months. These populations can then recover within 4 yr (Stańczykowska et al. 1975b).

Long-term abundance of *Dreissena* did not differ significantly between lakes with constant and variable populations (t-test, $p > 0.214$), despite the large differences in V between these two lakes types (t-test, $p < 0.002$) (Table 1). Lakes with constant populations did not occur in a different geographic area than those with variable populations. In fact, lakes with different population dynamics were sometimes connected by channels (Stańczykowska 1978).

Despite their proximity, lakes with constant and variable populations differed in their limnological characteristics (Tables 2, 3). DFA was very successful in distinguishing the two lake groupings (Table 4). The variables log(lake surface area), phosphate (PO_4), and calcium provided the most contrast between the two lake types in the multivariate analyses. Lakes with constant populations had larger surface areas, lower levels of calcium, and higher levels of phosphate than lakes with variable populations. None of the other morphometric, physical, or chemical variables were important in distinguishing the lake types.

We tested the accuracy of the discriminant function at classifying populations as constant or variable by comparing our a priori classification of the lakes with the predictions from the model. The function correctly classified 13 of the 18 original lakes for a 72.2% success rate. Another, more conservative method for estimating success of a DFA model is a cross-validation procedure using a jacknife technique. In this method the discriminant function is recalculated as many times as there are lakes, each time deleting only one lake from the dataset. Each lake is predicted to have either constant or variable populations using the model that was derived without use of that lake. The success rate using cross-validation was also 72.2%. The values of the discriminant function at the centroids of each group were 0.928 and −0.591 for lakes with constant and variable populations, respectively. The discriminant scores of four of the five misclassified lakes ranged from −0.16 to 0.31 and indicated that these lakes did not associate strongly with either lake type.

TABLE 2. Morphometric and physical characteristics of lakes with the two population types. Log(BASIN) is log(drainage basin area (km^2)), log(AREA) is log(surface area (km^2)), log(VOL) is log(lake volume (10^6 m^3)), Z_m is maximum depth (m), $\bar{Z}$ is mean depth (m), EPI is depth of the epilimnion (m), TEMP is maximum summer bottom temperature (°C), O_2 is minimum summer bottom oxygen ($mg \cdot L^{-1}$), and SECCHI is depth of Secchi disk visibility (m). MEAN is the average value of each variable for lakes of one population type, SD is standard deviation, MIN is the minimum value, and MAX is the maximum value.

Lake	log (BASIN)	log(AREA)	log(VOL)	Z_m	$\bar{Z}$	EPI	TEMP	O_2	SECCHI
				Lakes with constant populations					
Beldany	1.66	0.93	1.98	38.50	10.00	12.00	8.00	—	1.89
Boczne	1.71	0.28	1.20	15.00	8.60	10.00	16.00	0.00	3.00
Dargin	1.50	1.40	0.83	37.30	10.60	15.00	10.00	0.00	5.00
Dobskie	1.94	1.25	2.15	21.00	7.90	15.00	16.00	0.00	4.18
Niegocin	1.42	1.40	2.41	40.00	9.90	12.50	11.00	0.00	2.96
Sniardwy	2.18	2.04	2.82	25.00	5.80	25.00	16.00	6.00	2.33
Szymon	1.95	0.19	0.23	2.90	1.10	2.90	18.00	7.00	1.90
Mean	1.77	1.07	1.66	25.67	7.70	13.20	13.57	2.17	3.04
SD	0.271	0.660	0.930	13.903	3.331	6.633	3.823	3.371	1.173
MIN	1.42	0.19	0.23	2.90	1.10	2.90	8.00	0.00	1.89
MAX	2.18	2.04	2.82	40.00	10.60	25.00	18.00	7.00	5.00
				Lakes with variable populations					
Goldapiwo	—	0.99	2.03	25.70	10.88	9.50	12.87	1.50	3.00
Jagonde	1.95	0.97	1.92	34.00	8.70	6.00	8.00	0.00	1.70
Kuibyshev	6.12	3.81	4.76	40.00	8.90	11.00	7.80	8.32	3.75
Mokołajskie	1.05	0.66	1.75	27.80	11.00	9.00	6.40	2.20	1.72
Nehmitz	0.79	0.23	1.04	18.60	6.40	5.00	5.50	0.00	6.90
Stechlin	1.09	0.63	1.99	68.00	22.80	7.00	7.00	9.50	5.99
Stregiel	—	0.61	1.18	10.00	3.70	6.50	14.50	0.00	1.53
Taltowisko	1.86	0.51	1.66	36.50	14.00	12.00	6.40	3.00	4.50
Tałty	1.73	1.07	2.20	37.50	13.60	9.00	11.00	0.50	1.62
Wojnowo	1.71	0.28	1.07	16.50	6.30	16.50	16.00	0.25	1.10
Zabinki	—	−0.39	0.60	42.50	9.86	9.00	5.87	1.65	2.40
Mean	2.04	0.85	1.84	32.46	10.56	9.14	9.21	2.45	3.11
SD	1.704	1.065	1.094	15.759	5.103	3.233	3.738	3.357	1.954
MIN	0.79	−0.39	0.60	10.00	3.70	5.00	5.50	0.00	1.10
MAX	6.12	3.81	4.76	68.00	22.80	16.50	16.00	9.50	6.90

Discussion

Fluctuations in each component of a community potentially affect the dynamics of all other components, but these effects are manifest over a wide range of time scales. The life span of individuals of a species can affect the degree to which the consequences of population fluctuations are either propagated or attenuated through the system. For example, in limnetic systems the dynamics of the dominant herbivore, *Daphnia*, can follow a predator–prey cycle with its phytoplankton food base (McCauley and Murdoch 1987). This pattern is possible because the life cycle of both components is on the order of days to weeks. The pattern is attenuated at higher trophic levels because planktivorous and piscivorous fishes cannot respond numerically on similar time scales (McQueen et al. 1986). On the other hand, in systems where fish predators exert controlling influences on their prey the effects of their population fluctuations may be propagated through the system by cascading interactions (Hurlburt et al. 1971; Carpenter et al. 1985).

Like fish, *Dreissena* is a relatively long-lived consumer that can remove large percentages of the production of its food base. Life span of *Dreissena* (estimated from shell annuli) varies among lakes from 3 to 11 yr, with an average of 3–5 yr (Stańczykowska 1963). In lakes where *Dreissena* is abundant it can remove up to 62% of littoral primary production (Hamburger et al. 1990), up to 5–18% of total lake production (Stańczykowska 1975; Stańczykowska et al. 1975a), and can filter the entire volume of some lakes in a few days (Stańczykowska 1968). If *Dreissena* becomes abundant enough to have similar effects in North American lakes, the consequences of its population dynamics may affect many components of the limnetic comunity.

In habitats where *Dreissena* populations fluctuate, they may induce variability in several community components. *Dreissena* variability may affect phytoplankton dynamics which may, in turn, result in interannual fluctuations in zooplankton populations. Consumption of phytoplankton by *Dreissena* affects water clarity (Reeders et al. 1989), which indirectly affects the depth and density of macrophyte growth (e.g. Chambers and Kalff 1985; Dale 1986). If *Dreissena* becomes an important food source for fish and waterfowl, populations of these predators may also be affected by *Dreissena* fluctuations. The extent of economic damage caused by *Dreissena* would also vary with population size. Problems with biofouling, clogging of intake pipes, and encrusting of ships' hulls may be most severe during years of population peaks.

Understanding the causal mechanisms of *Dreissena* population dynamics is necessary for proper interpretation of our mathematical models. Spurious correlations and biases are most likely in small datasets. These must be identified before pre-

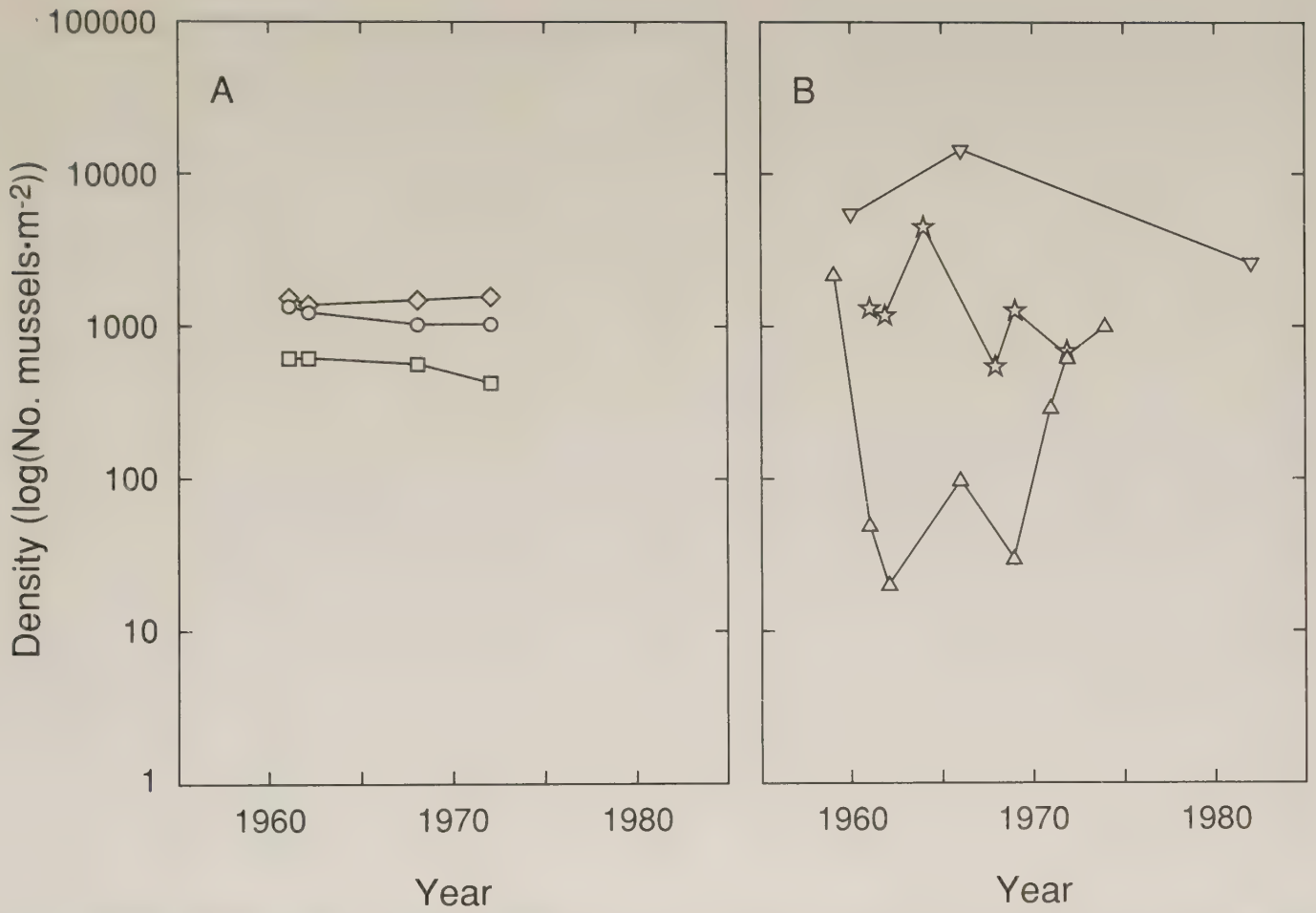

FIG. 1. *Dreissena* population dynamics in different European lakes from 1959 to 1985. Density is expressed as $\log_{10}$ (no. mussels·m $^{-2}$) in the zone of occurrence. (A) Lakes with constant populations of *Dreissena*, (B) lakes with variable populations. Circles are Lake Boczne, squares are Lake Sniardwy, diamonds are Lake Szymon, downwards triangles are Lake Stechlin, upwards triangles are Lake Mikołajskie, and stars are Lake Tałtowisko.

dictions can be confidently made for novel lakes. Our mathematical model should reflect the biological mechanisms that underlie population dynamics.

Population fluctuations may be caused by several different mechanisms. Predators such as fish (e.g. Plizska 1956; Biró 1974) and waterfowl (e.g. Borowiec 1983) can consume substantial proportions of *Dreissena* production. *Dreissena* also has several parasites including *Sphenophyra dreissenae* (Ciliata, Holotricha, Thigmotrichida) (Dobrańska 1958). However, at the population level, predators do not appear to be able to exert control over *Dreissena* (Stańczykowska 1990), and the effects of parasites are unknown (Stańczykowska 1977). A large population bottleneck for *Dreissena* appears to be high mortality in veliger and juvenile life stages (Walz 1978; Lewandowski 1982). Therefore, population fluctuations might be caused by interannual variation in factors such as wind mixing and temperature regime that may affect settling mortality and other aspects of larval recruitment variability.

In lakes with many diverse habitats, areas with reductions or extinctions of zebra mussels may be repopulated from elsewhere around the lake (habitat diversity hypothesis). Habitat diversity may be higher in larger lakes, just as it is in larger land masses (MacArthur and Wilson 1967). Therefore, lakes with larger surface areas should have more constant populations. This expectation is consistent with our model (Tables 2, 4).

Population fluctuations may also be a result of physiological stress caused by overcrowding, together with environmental variability (overcrowding hypothesis). Both the pattern of population fluctuations and physiological studies of mussel populations suggest that overcrowding is a factor in population crashes. *Dreissena* often reaches high abundance in the year prior to a precipitous population crash (Stańczykowska et al. 1975b; Lewandowski 1982). These crowded animals sometimes show signs of physiological stress (Stańczykowska 1977), perhaps as a result of resource limitation caused by their high abundance. They may weigh less for their body length in all size classes than animals grown under uncrowded conditions (Stańczykowska 1964). Environmental stresses such as a summer with reduced food supply or a winter with low oxygen may exacerbate physiological stress inducing population crashes. High abundance of *Dreissena* may have contributed to large winterkills in Lake Mikołajskie in 1959–60 and again in 1976–77.

Lakes that can support dense growths of *Dreissena* are more likely to have fluctuating populations (Stańczykowska et al. 1975b). We find that peak density of *Dreissena* is higher in lakes with fluctuating populations compared with lakes with constant populations (Table 1, *t*-test, $p < 0.044$). In another

TABLE 3. Chemical characteristics of lakes with the two population types. pH is average pH value, and the other variables are concentrations of nutrients and ions expressed as $mg \cdot L^{-1}$. PO_4 is phosphate, NO_3 is nitrate, Ca is calcium ion, Na is sodium, Cl is chlorine, K is potassium, and Fe is total iron. MEAN is the average value of each variable for lakes of one population type, SD is standard deviation, MIN is the minimum value, and MAX is the maximum value.

Lake	pH	PO_4	NO_3	Ca	Na	Cl	K	Fe
				Lakes with constant populations				
Beldany	7.80	0.14	0.10	41.00	4.75	9.23	2.14	0.08
Boczne	8.15	0.16	0.05	53.50	9.40	16.68	4.35	0.13
Dargin	8.15	0.10	0.07	41.50	6.50	12.58	3.33	0.00
Dobskie	8.15	0.07	0.08	40.67	6.60	12.66	3.31	0.00
Niegocin	8.15	0.18	0.14	49.40	8.94	17.09	4.32	0.13
Sniardwy	8.15	0.04	0.02	34.50	6.05	10.84	2.08	0.00
Szymon	8.15	0.16	0.05	53.50	9.40	16.68	4.35	0.13
Mean	8.10	0.12	0.07	44.87	7.38	13.68	3.41	0.07
SD	0.132	0.051	0.039	7.315	1.855	3.156	0.999	0.065
MIN	7.80	0.04	0.02	34.50	4.75	9.23	2.08	0.00
MAX	8.15	0.18	0.14	53.50	9.40	17.09	4.35	0.13
				Lakes with variable populations				
Goldapiwo	8.15	0.05	0.11	46.50	8.50	—	3.97	0.00
Jagonde	8.15	0.16	0.08	50.50	9.66	14.20	4.50	0.13
Kuibyshev	7.50	0.05	1.66	76.07	16.72	34.82	16.72	0.10
Mikołajskie	8.00	0.14	0.04	43.67	6.72	11.72	3.20	0.10
Nehmitz	7.80	0.00	0.04	47.92	—	—	—	—
Stechlin	7.80	0.01	0.01	50.00	—	—	—	—
Stregiel	8.00	0.05	0.11	50.17	7.55	11.00	3.90	0.01
Taltowisko	8.00	0.11	0.10	49.00	6.15	15.45	4.10	0.10
Tałty	8.00	0.11	0.11	49.00	6.15	15.45	4.10a	0.10
Wojnowo	8.15	0.16	0.05	53.50	9.40	16.68	4.50	0.13
Zabinki	8.38	0.01	0.43	54.00	7.05	—	3.13	0.00
MEAN	7.99	0.08	0.25	51.85	8.66	17.04	5.35	0.07
SD	0.233	0.061	0.483	8.539	3.292	8.105	4.292	0.054
MIN	7.50	0.00	0.01	43.67	6.15	11.00	3.13	0.00
MAX	8.38	0.16	1.67	76.07	16.72	34.82	16.72	0.13

TABLE 4. Coefficients of limnological variables used by the canonical discriminant function.

Variable	Coefficient
Ca	−0.115
PO_4	12.783
log(surface area)	0.666
Constant	3.788

study, we have found that *Dreissena* density tends to be higher in lakes with high calcium and low phosphate (C. W. Ramcharan, D. K. Padilla, and S. I. Dodson, unpubl. data). In this study, lakes with high calcium and low phosphate are more likely to have fluctuating populations. Together, these models suggest that populations may fluctuate more in lakes that can support high densities of *Dreissena* due, perhaps, to stresses caused by overcrowding.

Dreissena populations quickly recover from crashes. Within 1 yr, juveniles can settle in very high abundance on the empty shells left by the crash, and populations can grow by more than three orders of magnitude in 4 yr (compare Stańczykowska 1968 with Stańczykowska et al. 1975b for Lake Tałty). These rapid increases and declines are the characteristic pattern of lakes with the most variable populations. *Dreissena* populations that are more constant may be unable to overexploit the resources of their lakes, and may live in more constant limnological environments.

Our model is based solely on empirical correlations. It does provide some support for both the habitat diversity and the overcrowding hypotheses. Nevertheless, no explanation of population variability can be eliminated by this analysis. We suggest that experimental work should be used to test among these hypotheses to determine the mechanisms responsible for *Dreissena* population fluctuations.

We expect that *Dreissena* population dynamics will be affected by similar ecological processes in Europe as in North America. The lakes used in model development are fairly typical of temperate zone lakes in terms of morphometry, and physicochemical environments, although degree of cultural eutrophication may be higher in Europe than in North America. Physiological biases in North American *Dreissena* populations caused by evolutionary founder effects are also unlikely. North American populations show a wide genetic diversity and may be the result of either several separate introductions or one very large introduction (Hebert et al. 1989). Our model should, therefore, be directly applicable to most North American lakes.

Predictions for lakes such as the Laurentian Great Lakes may be more problematical because of their size and physical com-

plexity. Morphometric variables for the Great Lakes cover a much wider range of values, and in some cases are orders of magnitude greater than the data that we used for model development. The Great Lakes also have more complex patterns of water mixing and thermal stratification than the smaller lakes that we studied. Predictions from our model should be interpreted in light of the morphological and physical differences between the Polish lake set used for model development and the Great Lakes.

Our model predicts that *Dreissena* populations should be constant in all Great Lakes basins, including Green Bay, Saginaw Bay, Lake St. Clair, and western Lake Erie. The model's prediction is primarily influenced by the very large size of these water bodies. Local populations of *Dreissena* may fluctuate in abundance; however, it is unlikely that all or most colonies will stimultaneously decline in the many varied habitats found within each Great Lake.

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Appendix A

TABLE A.1. Sources of published data on lake morphometry (M), physical characteristics (P), chemical characteristics (C), and *Dreissena* abundance (D) that were used in this study. References are grouped into sources of European and North American data.

Data sources for European lakes

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Predation Risk, Prey Behavior, and Feeding Rate in *Daphnia pulex*

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Exposure to chemical exudates from the planktonic invertebrate predator *Chaoborus americanus* causes the prey *Daphnia pulex* to migrate upwards in the water column of laboratory experimental chambers. This behavior is an antipredator defense that results in spatial separation of *Daphnia* from *Chaoborus*, since this predator tends to remain near the bottom of the chambers. We test the effects of prey vulnerability, genotype, prior exposure to predators, and predator density on antipredator behavior of *D. pulex*. To study differences in vulnerability, we compared two genotypes of *Daphnia* that differed in their ability to produce a morphological defense (growth of neck spines) when exposed to *Chaoborus* chemical. Overall, behavioral responses to *Chaoborus* were stronger at higher predator densities. Juvenile *Daphnia* that are most vulnerable to *Chaoborus* predation had stronger responses than the less vulnerable adults. Neither genetically determined ability to produce neck spines nor phenotypic expression of neck spines affected strength of antipredator behavior. Vertical migration to avoid *Chaoborus* may have an ecological cost. Presence of *Chaoborus* reduced ingestion rates of juvenile *Daphnia* at low food levels (5×10^3 algal cells $\cdot$ mL^{-1}). Feeding experiments also showed differences in feeding characteristics of two different clones of *D. pulex*.

Des proies, *Daphnia pulex*, exposées en enceintes expérimentales de laboratoire à des exudats chimiques de *Chaoborus americanus*, un invertébré prédateur planctonique, se sont déplacées vers la partie supérieure des enceintes. Ce comportement constitue une défense contre les prédateurs qui se traduit par un isolement spatial des *Daphnia* et des *Chaoborus*, car ce prédateur a tendance à demeurer au fond des enceintes. Nous avons testé les effets de la vulnérabilité de la proie, du génotype, d'une exposition antérieure aux prédateurs et de la densité des prédateurs sur le comportement de défense des *D. pulex*. Afin d'étudier les écarts de vulnérabilité, nous avons comparé deux génotypes de *Daphnia* qui diffèrent de par leur capacité à produire une défense morphologique (croissance d'épines cervicales) après exposition aux substances chimiques de *Chaoborus*. De façon générale, les réponses comportementales à *Chaoborus* ont été plus importantes aux densités de prédateurs plus élevées. Les *Daphnia* juvéniles, qui sont les plus vulnérables à la prédation par *Chaoborus*, ont présenté des réponses plus fortes que les adultes, moins vulnérables. La capacité génétique de produire des épines cervicales ou l'expression phénotypique de ce caractère n'ont pas modifié l'importance du comportement de défense contre la prédation. La migration verticale faite pour éviter les *Chaoborus* pourrait cependant avoir un coût écologique. La présence de *Chaoborus* réduisait les taux d'ingestion des *Daphnia* juvéniles aux faibles teneurs en aliments (5×10^3 cellules d'algues $\cdot$mL^{-1}). Des expériences d'alimentation ont aussi montré l'existence d'écarts entre les caractéristiques d'alimentation de deux différents clones de *D. pulex*.

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Flexible antipredator strategies should theoretically provide the best balance between costs and benefits of defense, when danger from predation is variable and defense is costly (Lively 1986; Sih 1987). Studies with several species have verified that behavioral prey defenses can be induced when their benefits outweigh their costs at high abundance of predators (high predation risk) (e.g. Sih 1982; Dill and Fraser 1984; Werner and Hall 1988; Dodson 1989a; Kohler and McPeek 1989). Predation risk is, however, dependent on prey vulnerability as well as predator abundance, and more vulnerable animals may be at high risk even with relatively low predator abundance.

Populations of *Daphnia pulex* that inhabit small ponds can suffer heavy predation losses to *Chaoborus americanus*, against which they have evolved two defenses. The morphological defense is growth of a small, toothed, neck spine that reduces probability of ingestion by *Chaoborus* (Havel and Dodson 1984). The behavioral defense is avoidance of *Chaoborus* by vertical migration upwards in the water column (Dodson 1988). Upwards migration by *Daphnia* reduces mortality from *Chaoborus* because during daytime, *Chaoborus* has a strong tendency to avoid visually feeding fish by remaining in deeper, darker waters (Roth 1968). The behavioral defense in *Daphnia* has only recently been described (Dodson 1988) and its effectiveness as an antipredator defense is not yet understood (Liebold 1991). *Daphnia* also uses a third type of defense (life history defense); it increases intrinsic rate of population growth in response to presence of planktivorous fish (Dodson 1989b).

The *Daphnia–Chaoborus* system is ideal for the study of ecological and evolutionary aspects of antipredator adaptations (Dodson 1989a). All three defenses, the morphological, behavioral, and life history, are induced by chemical signals (kairomones) released in highest concentrations from actively feeding predators (S. I. Dodson, unpubl. data). Degree of expression of the morphological and behavioral responses

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depends to some extent on the age (developmental stage) of the *Daphnia*. The most vulnerable stages tend to show the greatest responses (Havel and Dodson 1985; Dodson 1988; Parejko 1990b).

In addition to the above phenotypic responses, degree of expression of the morphological and life history defenses varies among genetically different clones of asexually reproducing *Daphnia* (Parejko 1990a; Parejko and Dodson 1991; Spitze 1991). For example, some clones do not produce the morphological defense (are spine-incapable) even when exposed to high levels of *Chaoborus* chemical. Different *Daphnia* species also show differences in the strength of the vertical swimming response (Dodson 1988), suggesting the possibility of interspecific variation in behavior.

In this study, we examine how antipredator behavior and its costs are affected by three factors that may determine predation risk: prey vulnerability, predator density, and prey genotype. The prey are two different clones of the zooplankter *D. pulex* (Crustacea, Branchiopoda), and the predators are third and fourth instar larvae of *C. americanus* (Insecta, Diptera). In laboratory experiments, we test for effects of (1) life stage, (2) predator density, (3) genotype, and (4) prior conditioning on vertical swimming behavior in *Daphnia*. We also test the hypothesis that predator-induced changes in swimming pattern reduce feeding rate in *Daphnia*. For many animals, antipredator behavior has a cost in feeding rate when both are strongly related to movement patterns (e.g. Sih 1982; Dill and Fraser 1984; Werner and Hall 1988; Kohler and McPeek 1989).

We chose a spine-capable and a spine-incapable clone of *Daphnia* for these experiments because we were particularly interested in comparing the strengths of morphological and behavioral responses among clones. We expected to find the weakest behavioral defenses in prey that are either less vulnerable to predation (large *Daphnia*, or juveniles with neck spines) or prey that are incapable of growing neck spines. Spine-incapable prey cannot respond morphologically to *Chaoborus* chemical, and may also be unable to respond behaviorally. We also expected the strongest antipredator behavior in juvenile *Daphnia* that do not have neck spines, since these prey are most vulnerable to *Chaoborus* predation (Havel and Dodson 1984).

Methods

All experiments were conducted between December 1989 and September 1990. Both the spine-capable (SC) and spine-incapable (SI) clones of *D. pulex* originally co-occurred in Dead Dog Pond I (Havel 1985), Vilas County, Wisconsin. At the time of collection, *Chaoborus* abundance in Dead Dog Pond was low and clone SC was about four times more abundant than clone SI. All *Daphnia* used in these experiments descended from a single female of either clone. Third and fourth instar larvae (at least 80% fourth instar) of *C. americanus* (Cook 1956) were collected from Tender Bog, located at the University of Notre Dame Experimental Research Station, Gogebic County, Michigan. *Chaoborus* were kept in a cold room at 8–10°C for up to 4 wk before use.

We used filtered (Whatman No. 2 fiber filter), 4-d-aged tap water for all *Daphnia* cultures, *Chaoborus* collections, and experiments. *Daphnia* cultures were maintained on a mixture of *Chlamydomonas* sp., *Scenedesmus* sp., and other algae grown in batch cultures. Only *Chlamydomonas* sp. grown in continuous culture in Bold's Basal Medium was used for all experiments. "Unconditioned" *Daphnia* were cultured without presence of *Chaoborus*. "Preconditioned" *Daphnia* were exposed to *Chaoborus* chemical by suspending Nitex mesh bags containing 6–10 larval *Chaoborus* in their culture jars for 3 wk prior to use in experiments. The *Chaoborus* were fed 15–30 juvenile *Daphnia* (replaced as they were eaten) of the same clone that was in each culture jar. All *Daphnia* cultures were kept at room temperature (20–23°C).

Chaoborus chemical (kairomone) was prepared by placing 60 fourth instar larval *Chaoborus* in 3.0 L of filtered, aged tap water and feeding them at least 240 juvenile *Daphnia* (a mixture of clones) once each day for 4 d. By the third day, *Chaoborus* were approaching satiation, as several *Daphnia* remained in the tanks after 24 h. This culture was kept at 20–23°C.

Two size classes of *Daphnia*, juvenile and adult, were used in all experiments. *Daphnia* were sorted by size using 100- and 150-μm mesh for juvenile and adult animals, respectively. The smallest animals in the juvenile size class and the largest in the adult size class were then divided among experimental chambers. To ensure the same size distribution among all experimental units, we placed animals individually into a row of beakers, repeating the row until the required number of *Daphnia* were in each beaker.

To quantify *Daphnia* antipredator behavior, we conducted vertical migration experiments in an observation room that was painted with nonreflective black paint. The room was illuminated by a single bank of overhead fluorescent lights covered with blue and green gelatin filters to provide a more natural light colour. Room lights were kept on an 18 h light: 6 h dark cycle (lights on at 05:00, off at 23:00). Both *Daphnia* cultures and *Chaoborus* that were being used for kairomone generation were kept under the same light–dark cycle. All behavior and feeding experiments were conducted between 13:00 and 22:00.

Vertical position of *Daphnia* in the water column was quantified using 10 × 10 × 52 cm high, clear glass vertical migration chambers. The chambers were marked at water depths of 5, 10, 20, 30, 40, and 45 cm. During experiments, we recorded the number of *Daphnia* in each depth interval.

We exposed two body sizes of unconditioned and preconditioned *Daphnia* of both clones to four densities of *Chaoborus* (2 × 2 × 2 × 4 experimental design). Predator densities were 0, 0.1, 1.0, and 5.0 *Chaoborus* · L^{-1} and were achieved by dilution of predator-treated water. We used four replicates of experiments with unconditioned animals and three replicates for preconditioned animals. Into each experimental chamber we placed 20–40 juvenile and 20–30 adult *Daphnia* in 5.0 L of water containing 5×10^4 *Chlamydomonas* · mL^{-1}. The water was stirred to mix the *Daphnia* throughout the water column. Vertical distribution of Daphnia was counted by eye every hour for 4 h beginning 1 h after experiments were set up. We found that *Daphnia* would sink if exposed to even small changes in lighting regime; therefore, we always wore dark clothing in the observation room. Immediately after the second *Daphnia* count, kairomone was mixed throughout the tank by removing 1.25 L of water from each tank then refilling the tank with the appropriate dilution of *Chaoborus*-treated water. We also added either 5 or 25 fourth instar *Chaoborus* to the 1 and 5 *Chaoborus* · L^{-1} tanks, respectively. To ensure similar treatment for all tanks, we removed water from control tanks in which *Chaoborus* were absent and refilled the tanks with the same water.

To compare antipredator behavior and feeding rate, we conducted *Daphnia* grazing experiments in a temperature-controlled incubator at 20°C. We conducted all experiments in darkness, during the night period to which the animals had been

acclimated. Radioactively labelled algae were prepared by incubating 200 mL of 1×10^5 cells $\cdot mL^{-1}$ of log-phase growth *Chlamydomonas* in 50 μCi (1 μCi = 37 kBq) of radioactive $NaHCO_3$, under fluorescent lights for 2 d (Ramcharan and Sprules 1988).

Effects of experimental factors on feeding rate were measured in 1-L Fleakers (cylindrical flasks made by Corning Glass) in a $2 \times 2 \times 2 \times 2$ design. We used two clones of *Daphnia*, in two sizes, with two levels of *Chaoborus* chemical (the equivalent of either 0 or 5 *Chaoborus* $\cdot L^{-1}$). We also used two levels of food (low food was 5×10^3 cells $\cdot mL^{-1}$, and high food was 5×10^4 cells $\cdot mL^{-1}$). *Daphnia* in *Chaoborus*-present treatments had been preconditioned to *Chaoborus* for 3 wk. In each Fleaker, at least 60 juvenile and 60 adult *Daphnia* were placed in 1 L of experimental water and acclimated to experimental conditions of food, darkness, and temperature for 4 h. We then stirred 10 mL of ^{14}C-labelled algae into each chamber, allowed a 15-min feeding period, and filtered the animals onto Nitex screen. The animals were rinsed and then frozen with a block of frozen CO_2 for later analysis. A 2.0-mL algal sample from each Fleaker was filtered (Whatman GF/C) to check the level of radioactivity. An additional Fleaker containing only heat-killed juvenile and adult *Daphnia* was also incubated with radioactively labelled algae to provide an estimate of ^{14}C uptake that was not due to feeding.

To measure ingestion rate, the *Daphnia* were thawed and then solubilized by placing two adult or three juvenile animals from the same treatments into scintillation vials containing a mixture of 50% hydrogen peroxide and 50% perchloric acid (Bogdan et al. 1980) for 24 h. Animals were combined in scintillation vials in order to raise radioactivity to more easily measureable levels. Heat-killed *Daphnia* were combined in scintillation vials just as the experimental animals were. Since feeding rate is strongly dependent on body size (Peters and Downing 1984), body length of animals was measured (eyepiece micrometer on a Wild dissecting microscope) and only similar-sized animals were placed together in scintillation vials. Radioactivity of both zooplankton and algae was read by a liquid scintillation counter in 10 mL of EcoScint scintillation cocktail, using a quenching standard as a reference. Disintegrations per minute (DPM) for each *Daphnia* was calculated as

$$\text{DPM} \cdot \textit{Daphnia}^{-1} = \frac{(\text{DPM} \cdot \text{vial}^{-1} - \text{average DPM} \cdot \text{vial}^{-1} \text{ of heat-killed } \textit{Daphnia})}{\text{No. of } \textit{Daphnia} \cdot \text{vial}^{-1}}.$$

Ingestion rate was calculated as

$$\text{Ingestion rate (no. of cells} \cdot \textit{Daphnia}^{-1} \cdot \text{h}^{-1}) = \frac{\text{DPM} \cdot \textit{Daphnia}^{-1}}{\text{DPM} \cdot \text{mL}^{-1}} \times \frac{1}{0.25 \text{ h}} \times \text{no. of cells} \cdot \text{mL}^{-1}$$

where $DPM \cdot mL^{-1}$ is radioactivity of the algae from the experimental water.

Results

Results of behavioral and feeding experiments were analyzed by type II ANOVA (Systat, Wilkinson 1990). Bartlett's test (Sokal and Rohlf 1981) found no evidence for heteroscedasticity (nonhomogeneous variances) among our treatments ($p > 0.05$). *Chaoborus* densities of 5 predators $\cdot L^{-1}$ induced strong upwards shifts in the distribution of juvenile *Daphnia* in the water column (upward vertical migration response) and a weaker response in adult *Daphnia* (Fig. 1). Since vertical distributions were repeatedly determined (every hour) on the same experimental chambers, average depth at the end of the experiments might not have been independent of depth at previous time intervals. If initial depth differed significantly among treatments, then it should be used in an analysis of covariance with final depth as the dependent variable. Results of a four-way ANOVA (2 culture conditions × 2 clones × 2 body size classes × 4 *Chaoborus* levels) on initial position in the water column showed no statistically significant differences among treatments, and no significant interactions ($p > 0.05$). Statistical analyses were, therefore, only performed on final position in the water column.

Results of a four-way ANOVA showed no statistically significant four-way interactions; therefore, our final analysis was restricted to no more than three-way interactions (Table 1). We found a very strong overall effect of *Chaoborus* on vertical position of *Daphnia* in the water column ($p < 0.000$). *Chaoborus* densities of 1 and 5 predators $\cdot L^{-1}$ caused the strongest *Daphnia* responses. Animals that were preconditioned to *Chaoborus* (during culture) tended to remain higher in the water column, as did smaller animals, but neither trend was statistically significant ($p > 0.057$ and 0.067, respectively). A significant size class × *Chaoborus* interaction indicated that juveniles had a significantly stronger response to predators than adults ($p < 0.045$). The only statistically significant three-way interaction was preconditioning × clone × size class ($p < 0.016$): Adults of clone SC that had been exposed to *Chaoborus* chemical during development remained lower in the water column.

Results from feeding experiments were analyzed with separate three-way ANOVAs for juvenile and adult Daphnia (food density × clone × *Chaoborus*), rather than one four-way ANOVA (size class × food density × clone × *Chaoborus*) (Table 2). Three-way ANOVAs are easier to interpret. The large differences in clearance rate between juvenile and adult *Daphnia* cannot be compared using separate ANOVAs; however, these differences are already quite well known (Peters and Downing 1984). Feeding rate is directly related to body mass, and body mass increases as the square of length (e.g. dry weight (μg) = 10.674 × length $(mm)^{2.093}$, Lynch et al. 1986) (Table 3). Therefore, small differences in *Daphnia* length among our treatments may cause large, spurious differences in feeding rates. Any possible confounding effects of body size on feeding rate were removed by using weight-specific ingestion rate (ingestion rate/dry weight) in the three-way analyses of covariance.

Results of the two ANOVAs indicated several significant effects of food level, clone, and presence of *Chaoborus* chemical on juvenile and adult *Daphnia* (Table 2). One-way effects showed that ingestion rates of all *Daphnia* increased at high food levels and that both juveniles and adults of clone SI had higher ingestion rates than clone SC (Table 3). The significant food × clone interactions indicated that higher food levels did not increase feeding rate in clone SC as much as clone SI. Ingestion rate in clone SC was reduced by presence of *Chaoborus* chemical compared with clone SI (significant clone × *Chaoborus* interactions). The negative effects of *Chaoborus* chemical were strongest for juveniles of both clones at low food levels (significant food × *Chaoborus* interaction), while adults of clone SC at high food levels were most affected by *Chaoborus* chemical (significant three-way interaction).

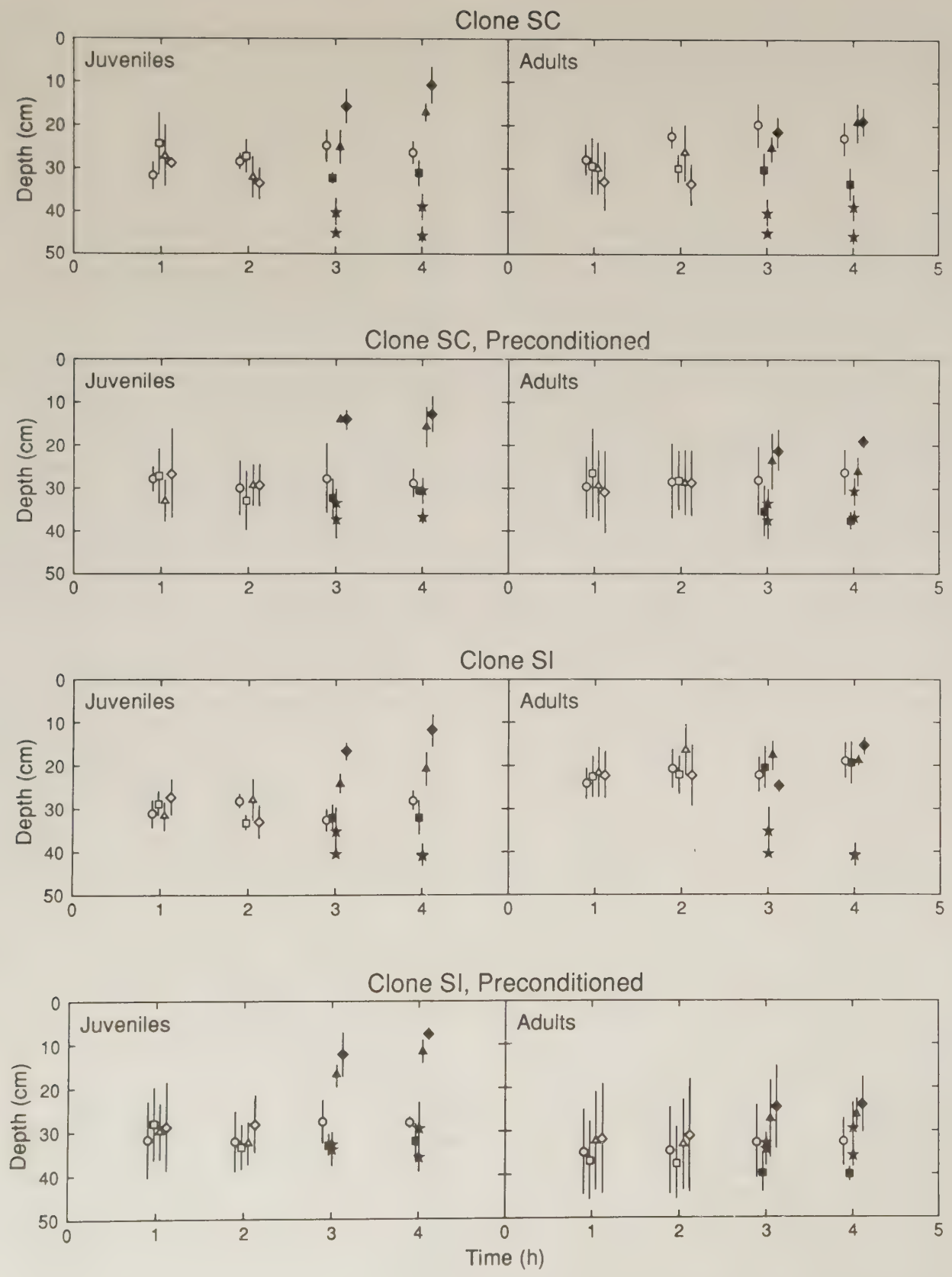

FIG. 1. Vertical distribution (± SE) of *Daphnia* in response to *Chaoborus*. The *x*-axis measures time from start of the experiment. The left panels show juvenile distribution and the right panels show adult distribution. Circles are controls with no *Chaoborus*, squares, triangles, and diamonds are 0.1, 1.0, and 5.0 *Chaoborus*·L^{-1}, respectively, and stars indicate the position of *Chaoborus*. Open symbols show vertical distribution before predators were added and filled symbols show the effects of *Chaoborus*.

TABLE 1. Results of model II ANOVA for vertical distribution. "Cond" indicates effects of prior exposure to *Chaoborus* chemical, "clone" is *Daphnia* genotype, "size" is size class (either juvenile or adult), and "*Chaoborus*" is predator density. *Statistically significant effect at $\alpha = 0.05$.

Source	Sum of squares	df	Mean square	*F* ratio	*p*
Cond	163.069	1	163.069	3.727	0.057
Clone	7.427	1	7.427	0.170	0.681
Size	150.755	1	150.755	3.445	0.067
Chaoborus	1834.460	3	611.487	13.974	0.000*
Cond × clone	30.881	1	30.881	0.706	0.403
Cond × size	88.174	1	88.174	2.015	0.159
Cond × *Chaoborus*	115.811	3	38.604	0.882	0.454
Clone × size	42.930	1	42.930	0.981	0.325
Clone × *Chaoborus*	124.960	3	41.653	0.952	0.419
Size × *Chaoborus*	366.695	3	122.232	2.793	0.045*
Cond × clone × size	266.717	1	266.717	6.095	0.016*
Cond × clone × *Chaoborus*	157.406	3	52.469	1.199	0.315
Cond × size × *Chaoborus*	80.314	3	26.771	0.612	0.609
Clone × size × *Chaoborus*	127.514	3	42.505	0.971	0.410
Error	3631.988	83	43.759		

TABLE 2. Results of model II ANOVA for weight-specific ingestion rate (no. of cells·μg^{-1} *Daphnia*$^{-1}$·h^{-1}) of juvenile and adult *Daphnia*. "Food" indicates effects of high or low food level, "clone" is *Daphnia* genotype, and "*Chaoborus*" is predator density. *Statistically significant effect at $\alpha = 0.05$.

Source	Sum of squares	df	Mean square	*F* ratio	*p*
Juveniles					
Food	1.666×10^7	1	1.666×10^7	199.439	0.000*
Clone	8.246×10^6	1	8.246×10^6	98.708	0.000*
Chaoborus	1.251×10^5	1	1.251×10^5	1.497	0.233
Food × clone	6.978×10^6	1	6.978×10^6	83.531	0.000*
Food × *Chaoborus*	6.800×10^5	1	6.800×10^5	8.140	0.005*
Clone × *Chaoborus*	3.534×10^5	1	3.534×10^5	4.230	0.042*
Food × clone × *Chaoborus*	1.323×10^5	1	1.323×10^5	1.583	0.211
Error	1.028×10^7	123	8.354×10^4		
Adults					
Food	4.605×10^7	1	4.605×10^7	161.605	0.000*
Clone	8.667×10^6	1	8.667×10^6	30.417	0.000*
Chaoborus	3.959×10^5	1	3.959×10^5	1.389	0.240
Food × clone	1.021×10^7	1	1.021×10^7	35.837	0.000*
Food × *Chaoborus*	3.649×10^5	1	3.649×10^5	1.281	0.260
Clone × *Chaoborus*	3.114×10^6	1	3.114×10^6	10.929	0.001*
Food × clone × *Chaoborus*	2.457×10^6	1	2.457×10^6	8.624	0.004*
Error	4.303×10^7	151	2.849×10^5		

Overall, the feeding rate experiments showed four trends: (1) clone SI had higher ingestion rates than clone SC, (2) high food levels enhanced feeding in clone SC more than in clone SI, (3) presence of *Chaoborus* chemical reduced ingestion rate of juveniles at low food levels, and adults of clone SC at high food levels, and (4) negative effects of *Chaoborus* chemical were strongest for clone SC.

Discussion

When danger from predation is constant, fixed defenses such as nocturnal foraging, or body armor, may be advantageous. However, when costs and benefits vary temporally and spatially over a wide range, flexible antipredator strategies should be favored (Lively 1986; Sih 1987). With flexible defenses, strength of expression of a prey defense should represent a balance between avoiding being eaten (benefit), and reductions in fitness (cost) caused by the defense. Factors which affect either probability of being eaten or cost of defenses would shift this balance and, therefore, affect strength of response to predators.

There are many aspects of *Daphnia*'s environment and life history that may affect the costs and benefits of defenses. In its natural habitat, *Daphnia* faces a predatory regime that varies spatially and temporally both in type and abundance of predators. For individual *Daphnia*, vulnerability to these predators changes as the animals grow, with the smallest instars usually suffering a much higher predation risk from invertebrate predators than larger instars (e.g. Pastorok 1980). A good example of the effects of prey vulnerability and predation risk on prey defense is the antipredator morphology used by *Daphnia* against *Chaoborus*. In the presence of *Chaoborus*, most strains of juvenile *D. pulex* grow a small neck spine (Krueger and Dodson

TABLE 3. Results of *Daphnia* feeding rate experiments. "Food" indicates low (5×10^3 cells·mL^{-1}) or high (5×10^4 cells·mL^{-1}) food levels, "clone" indicates *Daphnia* genotype (SC = spine-capable, SI = spine-incapable), and "*Chaoborus*" indicates presence or absence of *Chaoborus* chemical. *n* is the number of vials used in each treatment, "size" (mm) is average *Daphnia* body length, "dry weight" (μg) is calculated from length (Lynch et al. 1986), "ingestion rate" (no. of cells·*Daphnia*$^{-1}$·h^{-1} $\times 10^3$) is filtering rate (mL·*Daphnia*$^{-1}$·h^{-1}) multiplied by algal cell density, and weight-specific ingestion rate (no. of cells·μg^{-1}·*Daphnia*$^{-1}$·h^{-1}) is ingestion rate divided by dry weight. Means are reported with standard errors in parentheses.

	Food	Clone	*Chaoborus*	*n*	Size	Dry weight	Filtering rate	Ingestion rate	Weight-specific ingestion rate
Juveniles	Low	SC	Absent	16	0.93 (0.019)	9.16 (0.405)	0.21 (0.060)	1.23 (0.362)	139.69 (43.102)
			Present	17	0.89 (0.026)	8.41 (0.500)	0.03 (0.022)	0.17 (0.132)	16.90 (11.310)
		SI	Absent	17	1.00 (0.032)	10.67 (0.716)	0.25 (0.054)	1.53 (0.326)	139.55 (25.217)
			Present	16	1.04 (0.046)	12.00 (1.009)	0.19 (0.034)	1.16 (0.201)	97.51 (14.349)
	High	SC	Absent	18	1.00 (0.027)	10.60 (0.606)	0.07 (0.014)	3.29 (0.690)	311.03 (63.633)
			Present	16	0.95 (0.024)	9.59 (0.503)	0.07 (0.012)	3.38 (0.633)	349.55 (63.191)
		SI	Absent	16	1.10 (0.031)	13.27 (0.764)	0.30 (0.034)	15.07 (1.711)	1108.07 (82.819)
			Present	15	1.07 (0.024)	12.39 (0.600)	0.35 (0.034)	17.75 (1.729)	1481.90 (165.169)
Adults	Low	SC	Absent	20	2.40 (0.068)	67.62 (4.036)	2.10 (0.294)	12.57 (1.761)	181.53 (24.210)
			Present	19	2.47 (0.074)	72.43 (4.479)	1.67 (0.304)	10.01 (1.822)	146.28 (27.563)
		SI	Absent	20	2.51 (0.044)	74.01 (2.771)	1.34 (0.228)	8.05 (1.367)	110.36 (18.788)
			Present	20	2.54 (0.039)	75.42 (2.541)	1.72 (0.245)	10.32 (1.472)	137.64 (20.179)
	High	SC	Absent	20	2.61 (0.078)	81.15 (22.753)	1.68 (0.262)	85.53 (13.528)	1095.61 (192.744)
			Present	20	2.36 (0.061)	65.39 (3.385)	0.45 (0.095)	23.15 (4.841)	371.34 (76.129)
		SI	Absent	20	2.57 (0.044)	77.42 (2.819)	2.26 (0.288)	115.32 (14.695)	1540.95 (219.724)
			Present	20	2.67 (0.066)	84.42 (4.519)	3.13 (0.325)	159.60 (16.565)	1873.93 (141.352)

1981). This spine impairs the ability of *Chaoborus* to handle and ingest the *Daphnia* (Havel and Dodson 1984), but also causes a reduction in *Daphnia* fitness (*r*, intrinsic rate of population increase) (Havel and Dodson 1987; Walls and Ketola 1989; Black and Dodson 1990; Riessen and Sprules 1990). Both size of neck teeth and frequency of occurrence within a *Daphnia* population are reduced at lower densities of *Chaoborus* (lower predation risk) and at larger body sizes (lower vulnerability). Strength of the morphological response is also reduced by lowering probability of predation to each individual *Daphnia* by increasing density of other prey (A. R. Black, University of Wisconsin, Madison, WI, unpubl. data). Food levels that are low enough to constrain population growth shift the cost–benefit balance towards larger neck spines (H. P. Riessen, State University College at Buffalo, Buffalo, NY, unpubl. data). Finally, for a given set of conditions, response to predators may differ among genetic lines (Parejko and Dodson 1991).

All of the above ecological and genetic factors are already known to affect strength of expression of the morphological defense that *Daphnia* employs against *Chaoborus*. Our experiments test whether similar factors also affect behavioral defenses. The experiments presented here test (1) whether differences among prey in their vulnerability and risk of predation affect strength of expression of antipredator behavior, (2) whether antipredator behavior has a cost in terms of feeding rate, and (3) whether this cost is related to strength of the defense.

Vertical Migration Experiments

Results from our behavioral experiments generally support our hypothesis that strength of antipredator behavior is positively related to risk of predation. As in Dodson (1988), presence of elevated densities of *Chaoborus* (1.0 and 5.0 predators·L^{-1}) caused an overall upwards migration of *Daphnia*. In our experiments, upward migration often resulted in complete spatial separation of prey from the predator within 2 h. Smaller, more vulnerable *Daphnia* moved upwards sooner and stayed higher in response to *Chaoborus* than larger, less vulnerable animals. Juvenile *Daphnia* reacted so strongly to predator densities of 5.0 *Chaoborus*·L^{-1} that they would crowd into the meniscus formed between the top of the water column and the sides of our chambers.

We had expected that SI, the clone that could not be induced to produce neck teeth, would also show low induction of antipredator behavior. However, the similarity of migration patterns between the two different *Daphnia* clones indicates that capacity for the morphological and behavioral responses may be unrelated. Clone SI can either detect *Chaoborus* chemical, but is physiologically unable to grow neck teeth, or the morphological and behavioral kairomones are different, and SI can detect and respond to only the latter.

We expected those animals that were morphologically protected with a neck spine (preconditioned animals of clone SC) to have a weaker vertical migration response to *Chaoborus* than unspined animals, since spined animals are only half as vulnerable to predation as unspined animals (Havel and Dodson 1984). We found that, except for adults of clone SI, preconditioning had no overall effect on animals of either clone, and there was no evidence for long-term desensitization to *Chaoborus* chemical. Juveniles with neck teeth still used the added protection of the behavioral defense. Animals that migrate away from *Chaoborus* completely avoid predatory encounters for as long as *Chaoborus* is down near the bottom.

Feeding Experiments

Overall, the strength of antipredator behavior was related to cost of the defense as measured by ingestion rate. Juveniles had very strong behavioral responses to *Chaoborus* (Fig. 1; Table 1) and also suffered reductions in feeding rate at the low food level (Table 2). Adults had weak antipredator behavior, and in only one case (clone SC at the high food level) was ingestion rate substantially reduced by presence of *Chaoborus* chemical. *Chaoborus*-induced reduction in feeding rate may be caused by a reduction in frequency of either antennal or thoracic

leg beats, in order to produce a smaller hydromechanical signal to predators. Food may have been sufficiently abundant at higher food levels that animals would have a high feeding rate, even if they had less efficient, predator-induced feeding motions.

Our feeding experiments tested the relationship between vertical migration response and ingestion rate. A better indicator of the costs of defense is the intrinsic rate of population growth (r). It is possible that clone SC may be able to maintain a high r value, despite predator-induced reductions in feeding. On the other hand, *Daphnia* clones that grow neck spines in response to *Chaoborus* suffer reductions in r caused by slower development rate in juvenile instars (Havel and Dodson 1987; Walls and Ketola 1989; Black and Dodson 1990; Riessen and Sprules 1990). The reduction in feeding rate of juveniles that we found might be the causal mechanism for retarded development.

Our discovery of differences in feeding characteristics between two clones of *D. pulex* was unexpected. Clone SC had an overall lower ingestion rate than clone SI, and did not increase feeding rate at high food levels as much as clone SI. High food levels may clog *Daphnia* filtering appendages, reducing filtering efficiency and causing increased frequency of rejections of food particles (Porter and Gerritsen 1983: Porter and McDonough 1984).

In nature, there may be other costs to upwards vertical migration that we did not measure. For example, upper waters have higher abundance of predators such as notonectids (Dodson 1988) and are also better lit which facilitates feeding by planktivorous fish. When exposed to chemical exudates from these predators, some species of *Daphnia* migrate downwards in the water column (Dodson 1988). When two types of predators are simultaneously present, experiments on nonbehavioral defenses show that *Daphnia* can use a mixed strategy (life history changes and growth of neck spines) (A. R. Black, unpubl. data). Our study also shows evidence for use of mixed defensive strategies: juveniles of clone SC have the double protection of neck spines and antipredator behavior.

The effects of prey genotype may complicate attempts to measure the fitness costs of antipredator defenses. If genotypically different *Daphnia* vary in their physiological and life history characteristics (Spitze 1991; Tessier and Consolatti 1991), then risk–benefit models developed for one clone may not apply to others. On the other hand, if *Daphnia* genotypes represent local adaptations to strong environmental stresses, such as *Chaoborus* predation, then clones from similar ponds may have evolutionarily converged on similar risk–benefit functions. For example, Parejko and Dodson (1991) have found that genetic lines of *Daphnia* that were isolated from ponds with high abundances of *Chaoborus* tend to have stronger morphological responses to *Chaoborus* chemical than clones from low-*Chaoborus* ponds. In a similar study, we are currently testing the hypothesis that natural selection by *Chaoborus* has caused microevolution for enhanced antipredator behavior in high-predator ponds.

Acknowledgements

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A Skeletal Deformity of Northern Pike (*Esox lucius*) Related to Pulp Mill Effluents

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Lindesjöö, E., and J. Thulin. 1992. A skeletal deformity of northern pike (*Esox lucius*) related to pulp mill effluents. Can. J. Fish. Aquat. Sci. 49: 166–172.

Increased prevalences of a skeletal deformity affecting the skull of northern pike (*Esox lucius*) were related to three pulp mills in the Gulf of Bothnia, Sweden. At one mill the later disappearance of the disease, from an original prevalence of 35%, coincided with a marked improvement of effluent treatment. A typical deformed pike showed distinct upward bending of the jaws. The disease always affected the anterior part of the parasphenoid and the frontal bones. In the most severe cases the nasal, vomer, and medial rostral were also deformed. The degree of deformation was expressed as the ratio between two measurements made on the skull.

Une incidence accrue de déformations squelettiques du crâne de Grands brochets (*Esox lucius*) a été liée aux effluents de trois usines de pâte du golfe de Bothnie, en Suède. À l'une des usines, la disparition de la déformation, dont l'incidence antérieure était de 35 %, a coïncidé avec une amélioration marquée du traitement des effluents. La déformation type correspondait à une incurvation distincte vers le haut des machoires. La déformation touchait toujours la partie antérieure du parasphénoïde et les os frontaux. Dans les cas les plus sévères, le nasal, le vomer et le rostral médian étaient aussi déformés. Le degré de déformation était indiqué comme le rapport entre deux mesures du crâne.

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Several skeletal deformities have been observed in wild fish, affecting both the cranium and the vertebral column, e.g. pug head, shortening of the gill cover, vertebral compression, scoliosis, and lordosis (Möller and Anders 1986). In addition to genetic factors, deformities of both wild and cultured fish can be related to different environmental factors such as nutritional deficiency, parasitic infection, low pH, and exposure to heavy metals (Ferguson 1989). Furthermore, vertebral deformities have been induced experimentally by a large number of toxicants (Couch et al. 1977; Pragatheeswaran et al. 1987; Davis 1988) and industrial effluents (Bengtsson and Larsson 1986; Bengtsson et al. 1988b; Härdig et al. 1988). Consequently, skeletal deformities of fish have been implicated as possible indicators in pollution monitoring programmes (Bengtsson 1979; Vethaak and ap Rheinallt 1990). Few studies, however, have successfully established, on the basis of field evidence, a correlation between skeletal deformation and a specific pollution source, thus providing circumstantial evidence for a causal link (Vethaak and ap Rheinallt 1990). In contamination in southern California, chlorinated hydrocarbons and heavy metals were considered as chief suspects causing deformation of the gill rakers in barred sand bass (*Paralabrax nebulifer*) (Valentine 1975). In polluted areas of the River Rhine and its branches in the Netherlands, increased prevalences of pug heads and deformed fins of bream (*Abramis brama*) were suggested to be related to chemical pollution (Slooff 1982). Clearer evidence for a link with pollution was provided by a study of vertebral dysplasia in brown trout (*Salmo trutta*) attributable to a herbicide accidentally discharged in a Scottish stream (Wells and Cowan 1982). Furthermore, in a study of fourhorn sculpin (*Myxocephalus quadricornis*) in the Gulf of Bothnia, it was clearly demonstrated that pollution from a metal ore smelter was associated with skeletal deformities (Bengtsson et al. 1988a).

A multidisciplinary study concerning effects on the aquatic ecosystem of effluents from the Norrsundet bleached kraft pulp mill in Sweden began in 1982. The mill was partly rebuilt in 1982–83, with reconstruction and technical improvements of the processing technique, resulting in a markedly lower environmental load of effluent after 1984 (Södergren 1989; Lindesjöö and Thulin 1990). This provided an opportunity to study the prevalence of fish diseases in relation to improved water quality in the area affected by discharges from the Norrsundet pulp mill. In a previous study (Thulin et al. 1988), a high proportion of northern pike (*Esox lucius*) caught in the area of mill effluent had severe deformities of the jaw (Fig. 1) which were not observed in uncontaminated areas. Thus, we developed a morphometric index to monitor this jaw deformity in pike from areas affected by pulp mill effluents and from reference areas in 1988 and 1990; finally, material from a previous study (Thulin et al. 1988) was reanalysed in an attempt to detect temporal changes.

Materials and Methods

Pike from areas influenced by effluent from the Norrsundet, Iggesund, and Husum pulp mills were collected: except for the

FIG. 1. Northern pike caught at Iggesund pulp mill, Gulf of Bothnia, with severe pike jaw deformity (PJD index = 0.27).

FIG. 2. Positions of pulp mills (Norrsundet, Iggesund, and Husum) and reference areas (Simpevarp, Forsmark, Härnösand, and Råneå) sampled in the Baltic Sea and Gulf of Bothnia.

pulp mill outlet, no other pollution sources existed in the areas. Four reference areas without any local industrial activity were also studied: Simpevarp, Forsmark, Härnösand, and Råneå (Fig. 2). All areas are situated in sheltered archipelagos with salinity decreasing in a northerly direction from 6‰ at Simpevarp to 2‰ at Råneå. All the mills produce bleached chemical pulp with total production/effluent discharge (chemical oxygen demand) in tonnes per year of 240 000/13 500 (Norrsundet, 1988), 275 900/20 500 (Iggesund, 1988), and 590 000/46 400 (Husum, 1989). There are clear gradients of pulp mill discharge concentration, with the highest contamination closest to the mills. The following dilution factors have been calculated at the discharge point at the different mills: 166× (Norrsundet), 25× (Iggesund), and 190× (Husum) (Liljeblad 1985; Håkansson 1987; Södergren 1989). The effluent area and the effluent characteristics at Norrsundet were described in detail by Lindesjöö and Thulin (1990).

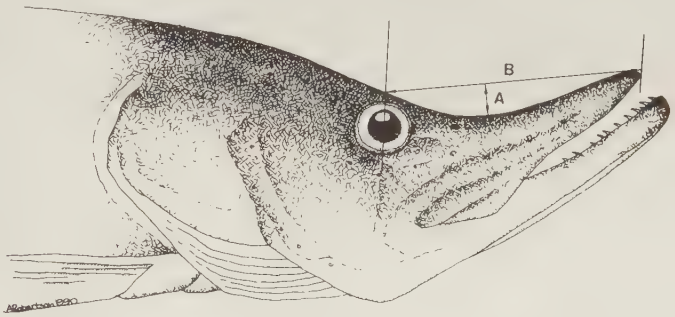

FIG. 3. Measurements taken to calculate the skull deformation index (A/B) for northern pike.

Pike were caught with fyke and gill nets at Simpevarp (n = 158), Forsmark (n = 284), Norrsundet (n = 86), and Iggesund (n = 45), during April–June 1988. Pike at Härnösand (n = 23), Husum (n = 14), and Råneå (n = 33) were sampled 2 yr later during the same months. This is the spawning/postspawning period for pike when they stay in very shallow waters. Sampling at the pulp mills was conducted within a distance of 6 km from the discharge point. All pike were then examined for external lesions. The sex and length were noted, and photographs were taken to show a lateral view of the skull. All skulls of pike caught in 1988 were boiled to remove various bones,

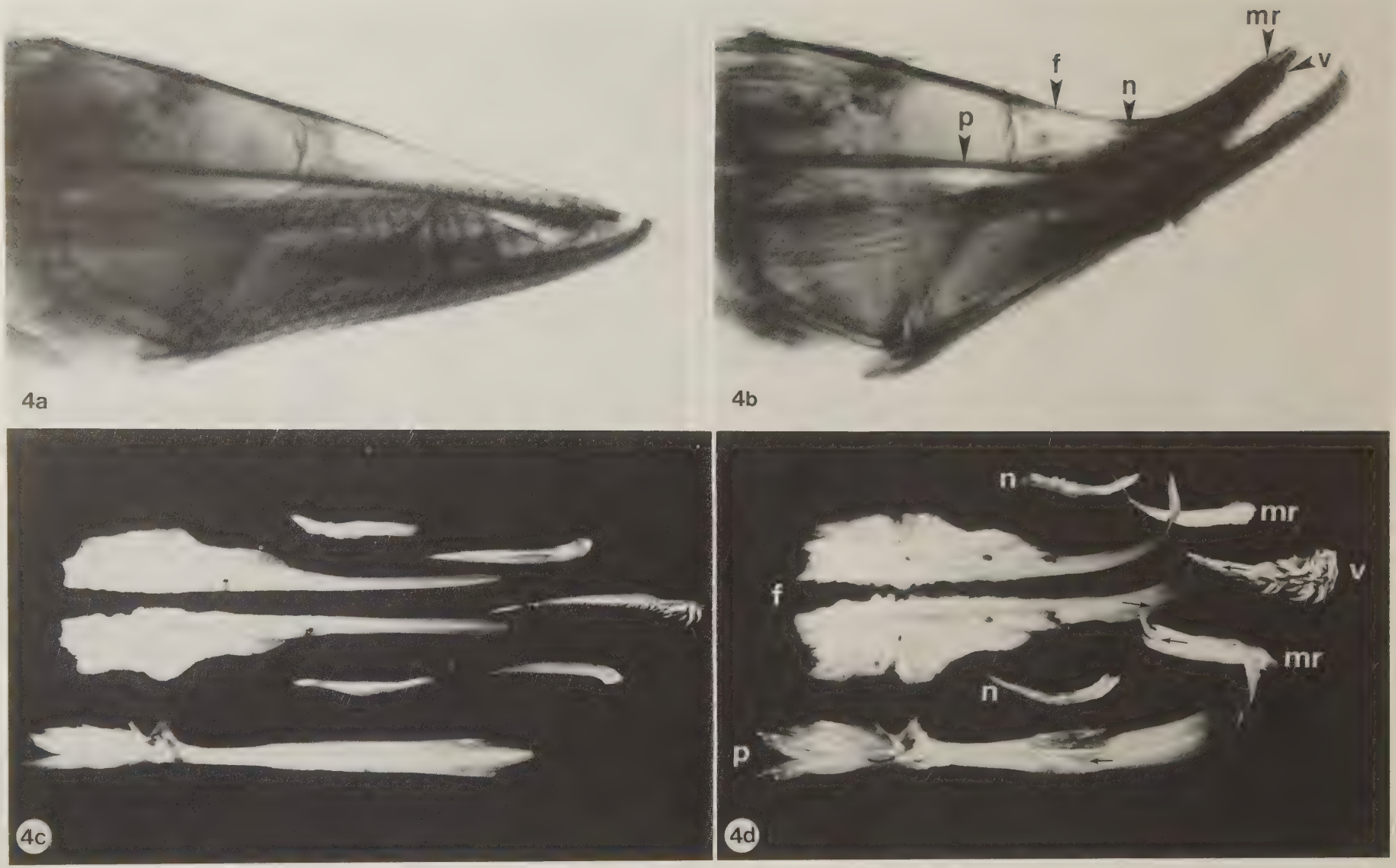

FIG. 4. X-ray photographs of northern pike (a) without skull deformation and (b) with severe PJD (index = 0.17) caught at the Norrsundet pulp mill, Gulf of Bothnia, in 1984. Photographs of (c) bones from the upper jaw of an unaffected northern pike and (d) bones from the upper jaw of a northern pike, caught at the Iggesund pulp mill, Gulf of Bothnia, with severe PJD. Arrows show areas with ''porous/loose'' appearance. f = frontal, p = parasphenoid, n = nasal, mr = medial rostral, v = vomer.

TABLE 1. Data on the skull deformation index, age, and length of northern pike at different pulp mill and reference areas in the Baltic Sea and Gulf of Bothnia, Sweden.

	Reference areas				Pulp mill areas		
	Simpevarp	Forsmark	Härnösand	Råneå	Norrsundet	Iggesund	Husum
Deformation index							
Mean	0.021	0.021	0.020	0.028	0.025	0.037	0.041
Min.	0.008	0.004	0.012	0.016	0.011	0.008	0.022
Max.	0.040	0.077	0.030	0.051	0.050	0.266	0.085
SD	0.005	0.008	0.005	0.006	0.008	0.038	0.016
No. of fish	158	284	23	33	86	45	14
Age (yr)							
Mean	5.2	4.7	—	—	4.5	5.7	—
Min.	2	2	—	—	2	3	—
Max.	13	11	—	—	8	12	—
SD	1.8	1.6	—	—	1.1	1.9	—
No. of fish	155	260	—	—	83	42	—
Length (mm)							
Mean	526	560	572	632	561	626	563
Min.	336	341	330	150	326	410	440
Max.	799	965	740	890	741	950	850
SD	92	122	96	148	89	121	103
No. of fish	158	278	23	33	84	44	14

and the pterygoid bone was dissected for age determination (Segerstråle 1948). Since only a very weak correlation between the deformation and age was found in the material from 1988, no age determination was made on pike sampled at Härnösand, Husum, and Råneå in 1990 (see below). In addition, all skull bones of the pike caught at Iggesund pulp mill were dissected and examined for possible deformities, as were the skull bones of 60 specimens from the reference area at Forsmark.

To express the degree of jaw deformation, the distance between the centre of the eye and the anterior extremity of the upper jaw (B) was drawn on an 18 × 24 cm print of the lateral view of the skull (Fig. 3) and measured to the nearest millimetre with a ruler. The maximum perpendicular distance (A) between line B and the dorsal surface of the upper jaw was then measured to the nearest 0.01 mm using a dissecting microscope and ocular micrometer. The degree of jaw deformation was expressed in terms of the index A/B.

A chemical analysis of the anterior part of the frontal bone of pike affected by the pike jaw deformity (hereby named PJD) was performed. A total of 14 pike, age 2–8 yr with a deformation index exceeding 0.06, were analysed. As control, 28 pike within the same age interval as the affected pike were analysed from the reference Forsmark. Calcium was determined by the method developed for the Tecator 5020 Flow Injection Analyzer (TECATOR 1982). Phosphorus was analysed according to the method described by Fiske and Subbarow (1925), collagen according to the method of Flanagan and Nichols (1962), and proline and hydroxyproline according to the methods of Troll and Lindsley (1955) and Woessner (1961).

Results

Description of the Deformity

A pike affected by PJD showed a characteristic upward bending of the anterior part of the parasphenoid and the frontal bones. Varying degrees of deformation were found, from slight to severe. In the most severe cases the nasal, vomer, and medial rostral were also deformed (Fig. 4b, 4d) by comparison with normal pike (Fig. 4a, 4c). Two of the affected pike from Iggesund showed a "porous/loose" appearance of most parts of the parasphenoid, frontal, nasal, vomer, and medial rostral (Fig. 4d). Furthermore, the distal parts of the vomer and medial rostral were missing. In the single pike with PJD from the reference areas, i.e. the one from Forsmark (see below), a similar but weaker deviation of the central part of the frontal and parasphenoid bones was found.

No external lesions were observed with PJD, except some incidental cases of ulceration and haemorrhage. Although the condition factor was not measured directly, no obvious sign of weight loss was found in association with PJD.

Relation with Age, Length, and Sex

All fish sampled were aged 2 yr or older (Table 1). Only negligible correlations were found between the index of deformation and age and length (Table 2). However, at Iggesund, a significant increase of the deformation index ($p < 0.05$, Mann–Whitney U-test) was found among the females (Table 3). No significant deviations of the deformation index between the sexes were found at the other areas.

Measurement of Deformation

To evaluate the prevalence of pike with deformities, pike having an index higher than the mean value of all reference areas plus 3 standard deviations, i.e. 0.0431 ($p < 0.0015$), were defined as being affected by PJD. The prevalences of PJD at the reference areas varied between 0.0 and 3.0%, while increased prevalences were found at Iggesund and Husum pulp mills, 18 and 29%, respectively (Fig. 5). However, at Norrsundet pulp mill, after improvement of effluent treatment, the prevalence was only 1.2%. The variation between the areas was significant ($p < 0.0001$, chi-square). In addition, the highest mean values of the deformation index were also found at Iggesund and Husum pulp mills (Table 1). These mean values

TABLE 2. Correlation coefficients of the skull deformation index with age and length of northern pike at different pulp mill and reference areas in the Baltic Sea and Gulf of Bothnia.

	Reference areas				Pulp mill areas		
	Simpevarp	Forsmark	Härnösand	Råneå	Norrsundet	Iggesund	Husum
Age (yr)							
r	0.072	0.182	—	—	0.180	0.271	—
p	0.372	0.003	—	—	0.103	0.082	—
No. of fish	155	260	—	—	83	42	—
Length (mm)							
r	0.099	0.151	0.091	0.210	0.163	0.290	0.346
p	0.214	0.012	0.679	0.242	0.138	0.056	0.226
No. of fish	158	278	23	33	84	44	14

TABLE 3. Data on the skull deformation index of males and females of northern pike at different pulp mill and reference areas in the Baltic Sea and Gulf of Bothnia. The data are presented in the order male/female; *significantly different $p < 0.05$, Mann–Whitney *U*-test).

	Reference areas				Pulp mill areas		
	Simpevarp	Forsmark	Härnösand	Råneå	Norrsundet	Iggesund	Husum
Mean	0.021/0.021	0.020/0.022	0.019/0.021	0.025/0.029	0.024/0.026	0.027/0.047*	0.041/0.043
Min.	0.007/0.032	0.004/0.005	0.012/0.027	0.016/0.021	0.011/0.011	0.008/0.015	0.022/0.038
Max.	0.032/0.040	0.046/0.077	0.028/0.030	0.035/0.051	0.050/0.041	0.076/0.266	0.086/0.051
SD	0.005/0.005	0.008/0.009	0.006/0.005	0.005/0.007	0.008/0.008	0.015/0.051	0.018/0.007
No. of fish	85/73	146/132	12/11	11/22	45/39	23/22	11/3

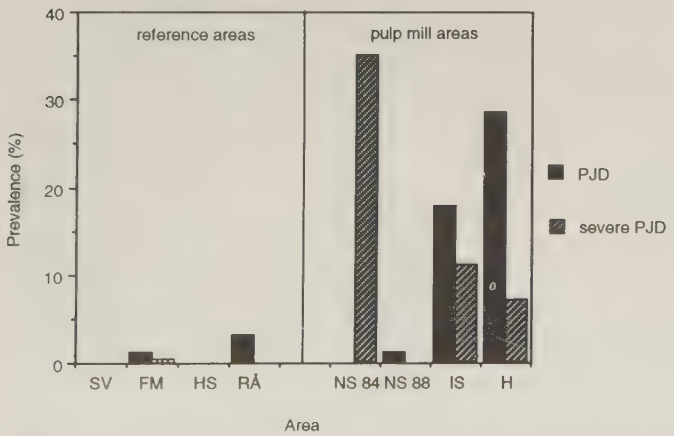

FIG. 5. Bar chart showing prevalences of PJD (index > 0.0431) and severe PJD (index > 0.06) in northern pike at pulp mill and reference areas in the Baltic Sea and Gulf of Bothnia, Sweden. SV = Simpevarp, FM = Forsmark, HS = Härnösand, RÅ = Råneå, NS 84 = Norrsundet in 1984, NS 88 = Norrsundet in 1988 (after improvement of effluent treatment), IS = Iggesund, H = Husum.

include pike with and without PJD. Variation of the mean index between areas was significant ($p < 0.0001$, Kruskal–Wallis).

Previous Observations at Norrsundet (1984)

At Norrsundet pulp mill in 1984, before improvement of effluent treatment, 35% of a sample ($n = 40$) of northern pike caught in the effluent area showed a marked deformation of the jaw. This deformity was not observed in samples of pike ($n = 1260$) caught at that time in reference areas in the Baltic Sea and Gulf of Bothnia (Thulin et al. 1988). It is clear that these pike were affected by the same pathological condition as described in this paper. On the basis of pictures and bones saved from the Norrsundet material in 1984, specimens originally registered as being affected by the deformity showed macroscopically pronounced deformation with a deformation index exceeding 0.06 (termed here "severe PJD") and a highest value of approximately 0.17 (Fig. 4b). To compare the occurrence of severe PJD at Norrsundet in 1984 with the 1988 and 1990 samples, the prevalences of severe PJD were recalculated. At Norrsundet pulp mill in 1988, after improvement of effluent treatment, no pike was found with severe PJD. However, at Iggesund and Husum pulp mills, pike with severe PJD were found with prevalences of 11 and 7%, respectively. Only one fish (0.4%) was found with "severe PJD" at one of the reference areas, i.e. Forsmark (Fig. 5). Differences between these samples were significant ($p < 0.0001$, chi-square).

Chemical Analysis

The content of phosphorus in the control group was found to be related to age. Thus, the material was divided into two age groups: pike 2–4 yr and pike 5–8 yr. Significantly increased contents of both phosphorus and calcium were found in the older age group of pike with PJD compared with the control of the same age group (Table 4). No significant changes were found in the content of collagen (PJD: 217.4 ± 31.9 mg/g bone, control: 215.6 ± 44.1 mg/g bone), proline (PJD: 147.7 ± 7.9 mg/g collagen, control: 147.0 ± 9.8 mg/g collagen), and hydroxyproline (PJD: 78.7 ± 3.8 mg/g collagen, control: 80.2 ± 6.4 mg/g collagen). Furthermore, no sexual dimorphism could be demonstrated.

Discussion

A clear pattern of disease occurrence was found, with the highest prevalences of PJD at the pulp mills. With the exception of a single specimen, pike with severe PJD were not found in the reference areas. In a Finnish study of fish diseases in the areas of Åland archipelago and the Archipelagic Sea, none of

TABLE 4. Data (mean, SD, and no. of pike analysed) on the chemical composition of the frontal bone of northern pike affected by severe PJD. *Significantly different from respective control ($p < 0.05$, Mann–Whitney U-test).

	Calcium (mg/g bone)		Phosphorus (mg/g bone)	
Age (yr)	Control	Pike with PJD	Control	Pike with PJD
≤4	246.6 ± 6.2 (17)	242.1 ± 15.3 (7)	127.2 ± 2.9 (17)	127.9 ± 6.3 (7)
≥5	248.0 ± 6.1 (11)	259.6 ± 8.2 (7)*	124.1 ± 3.1 (11)	127.9 ± 3.8 (7)*

4320 pike examined between 1983 and 1985 were found with any deformity resembling PJD described in this paper (T. Wiklund, Institute of Parasitology, Åbo Akademi, Porthansgatan 3, 20500 Åbo, Finland, pers. comm.). This indicates that severe PJD occurs in very low prevalences (<1%) in areas unaffected by local pollution. In addition, the highest mean values of the deformation index in this study were recorded at Iggesund and Husum pulp mills. Furthermore, a very high prevalence (35%) of severe PJD at Norrsundet pulp mill in 1984 dropped to zero 4 yr later, coinciding with a marked improvement of effluent treatment at this mill. This corresponds with the sudden disappearance of another fish disease, fin erosion in perch (*Perca fluviatilis*), which was also found in the same effluent area (Lindesjöö and Thulin 1990). More than 60% of the perch had acute fin erosion in 1984. After 1984, however, no new outbreaks of fin erosion occurred, which coincides with the decreased load of effluent from the mill as well as with the disappearance of PJD in pike. The study of Lindesjöö and Thulin (1990) clearly indicated that the original outbreak of fin erosion was caused by the bleached kraft mill effluents from the pulp mill, a determining factor for this conclusion being the sedentary behaviour of perch. Tagging experiments in the Baltic Sea and the Gulf of Bothnia have shown that pike are even more sedentary than perch. The maximum distance covered has been shown not to exceed 3 km for 90% of the pike in a particular population (Karås and Lehtonen 1990). Homing behaviour was also shown to be very strong for pike. This implies that the affected pike developed PJD at the capture locations.

The etiology of PJD has not yet been established. The pattern of prevalence suggests that a toxic substance or combination of different substances in bleached kraft mill effluents may cause the disease. However, secondary effects such as oxygen deficiency arising from the discharge of effluents should not be overlooked. A heavy organic load in the Norrsundet effluent regularly caused oxygen saturation to fall to 30–40% in the central part of the inner effluent area during the early 1980's (Grahn and Sangfors 1984). Similar conditions are known to occur in the Iggesund effluent area where deposits of fibres and other organic material can locally cause low oxygen levels (Ekelius et al. 1984). Anoxic sediments rich in hydrogen sulphide have also been found during the last 4 yr in the effluent area at the Husum pulp mill (Eriksson and Nissling 1988).

Pike are extremely tolerant of low oxygen and can survive oxygen levels as low as 0.3 mg/L (Casselman 1978). They may therefore be able to tolerate the low oxygen levels in effluent areas, but at the cost of developing the skeletal deformity in question. This hypothesis is strengthened by a previously known case of a jaw deformity of pike which was, however, not scientifically described. At the end of May 1971 in Lake Norrviken, situated 20 km north of Stockholm, Sweden, four of nine pike caught by private fishermen had jaw deformities. Photographs taken of these pike, published in a local paper, show identical symptoms to severe PJD described here. The finding of deformed pike was also made by other fishermen in the lake during the spring, indicating that a large part of the pike population was affected by the deformity. The lake was very eutrophic due primarily to effluents from a yeast factory. A limnological study of the lake during the 1960's showed that most of it was completely deoxygenated during the period following the regular ice-breaking in April every year. Furthermore, large amounts of hydrogen sulphide were produced in the anaerobic layers (Ahlgren 1967). After 1970 the use of the lake as a recipient for factory effluent ceased; no new cases of pike deformities have been reported since.

In a study of the effects of pulp mill effluents on vertebrae of fish, perch from Norrsundet pulp mill effluent in 1985 were found with increased levels of collagen and phosphorus, while calcium was decreased (Bengtsson 1988). In a similar study, it was found that the vertebrae of fourhorn sculpin from the effluent area of a metal ore smelter had an increased level of phosphorus (Mayer et al. 1988). Although it is not possible to make a direct comparison between these studies and the pike study presented in this paper due to different fish species examined and different parts of the skeleton sampled, the increased levels of phosphorus found in all cases might be an effect of the exposure from pulp mill and ore smelter effluents. However, it can be concluded that the increased levels of both calcium and phosphorus of pike affected by PJD show that no decalcifying process was involved at the time of sampling.

In conclusion, the present study shows a correspondence between increased prevalences of PJD and pulp mill effluents. At the Norrsundet pulp mill the high prevalence of PJD, as well as that of other fish diseases, disappeared after a marked improvement of effluent treatment. This evidence strongly suggests a causal relationship between pulp mill effluents and skeletal deformities.

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Role of Temperature, Dissolved Oxygen, and Backwaters in the Winter Survival of Freshwater Drum (*Aplodinotus grunniens*) in the Mississippi River

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Annual winter impingement of large numbers of freshwater drum (*Aplodinotus grunniens*) on screens of a power station intake on the Mississippi River led to an investigation of the cause of impingement. Impingement occurred most abundantly in winter and early spring and primarily involved juvenile fish. Pronounced drift of moribund and dead fish was found in the main channel above and below the power station in late winter. Laboratory studies indicated that juvenile freshwater drum became disoriented, incapacitated, and suffered increased mortality as water temperature dropped to 1°C and below. In winter and early spring, temperatures were 0°C in the main and side channels of the river, but pockets of water above 1°C existed in some backwaters. Dissolved oxygen concentrations declined through the winter, becoming very low in some backwaters. An aggregation of fishes including freshwater drum was observed in the warmer backwaters. Variations in river flow and dissolved oxygen depletion in some backwaters were postulated to cause periodic disruption of the thermal refuges and an associated appearance of incapacitated and dead juvenile freshwater drum in the drift. If man-induced changes to the river eliminate backwater winter refuges, the ichthyofauna of the river could ultimately be altered.

Des quantités importantes de malachigans (*Aplodinotus grunniens*) viennent se heurter à chaque hiver aux grilles de protection de la prise d'eau d'une station hydroélectrique de la rivière Mississippi et une étude visant à en déterminer les causes a été réalisée. Le phénomène se produit surtout en hiver et au début du printemps et intéresse généralement des poissons juvéniles. Des quantités appréciables de poissons moribonds ou morts ont été décelés, à la fin de l'hiver, dérivant dans le chenal principal tant en amont qu'en aval de la station. Des études en laboratoire ont montré que les malachigans juvéniles devenaient désorientés, incapables de réagir et présentaient un taux de mortalité accru lorsque la température tombaient à 1°C ou moins. En hiver et au début du printemps, des températures de 0°C ont été notées dans le chenal principal et les chenaux latéraux du cours d'eau, mais des zones à température supérieure à 1°C ont été décelées dans des zones de contre-courant. Les teneurs en oxygène dissous diminuaient tout au long de l'hiver pour tomber à de très faibles valeurs dans certaines zones de contre-courant. Un groupe de poissons comprenant des malachigans a été décelé dans ces eaux plus chaudes. On formule l'hypothèse que les variations de l'écoulement et l'absence d'oxygène dissous dans certaines zones de contre-courant ont pour effet de perturber de façon périodique les refuges thermiques et que cela correspond à l'apparition de malachigans juvéniles incapables de réagir, ou morts, dans les eaux d'écoulement. La réalisation de travaux ayant pour effet de faire disparaître les refuges d'hiver situés dans les zones de contre-courant pourrait éventuellement perturber l'ichthyofaune du cours d'eau.

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During winter, large numbers of fishes have been observed by station fishery biologists impinged on the cooling water intake screens of the Quad Cities Nuclear Station (QCS), located at River Mile 506.4 (RM, above the confluence with the Ohio River) on Pool 14 of the Mississippi River. Although several species were affected, the freshwater drum (*Aplodinotus grunniens*) was selected for detailed study because it is one of the most abundant species in the fish communities of large rivers of the central United States and has the greatest latitudinal range of North American freshwater fishes (Barnickol and Starret 1951; Scott and Crossman 1973). In the section of river adjacent to Illinois, freshwater drum greater than 250 mm total length are harvested commercially, and the mean annual harvest from Pool 14 of 11 812 kg from 1980 through 1985 ranks third among commercial species taken from that pool (UMRCC 1982–85). Estimated standing stock of freshwater drum in Pool 14 greater than 220 mm ranged between 99 and 309 fish·ha^{-1} for the same period (LMS 1986).

Observations of freshwater drum near the surface at the power station intake indicated that these fish were physically incapacitated, incapacitation being characterized by disoriented and uncoordinated swimming behavior with alternating periods of quiescence (L. Bodensteiner, pers. obs.). We hypothesized that small freshwater drum were intolerant of some environmental factor present in the main channel of the Mississippi River during winter.

Our first objective was to determine the spatial occurrence of incapacitated freshwater drum: were they limited to the vicinity of the power plant, confined to Pool 14, or did they occur on a widespread basis with incapacitated fish drifting

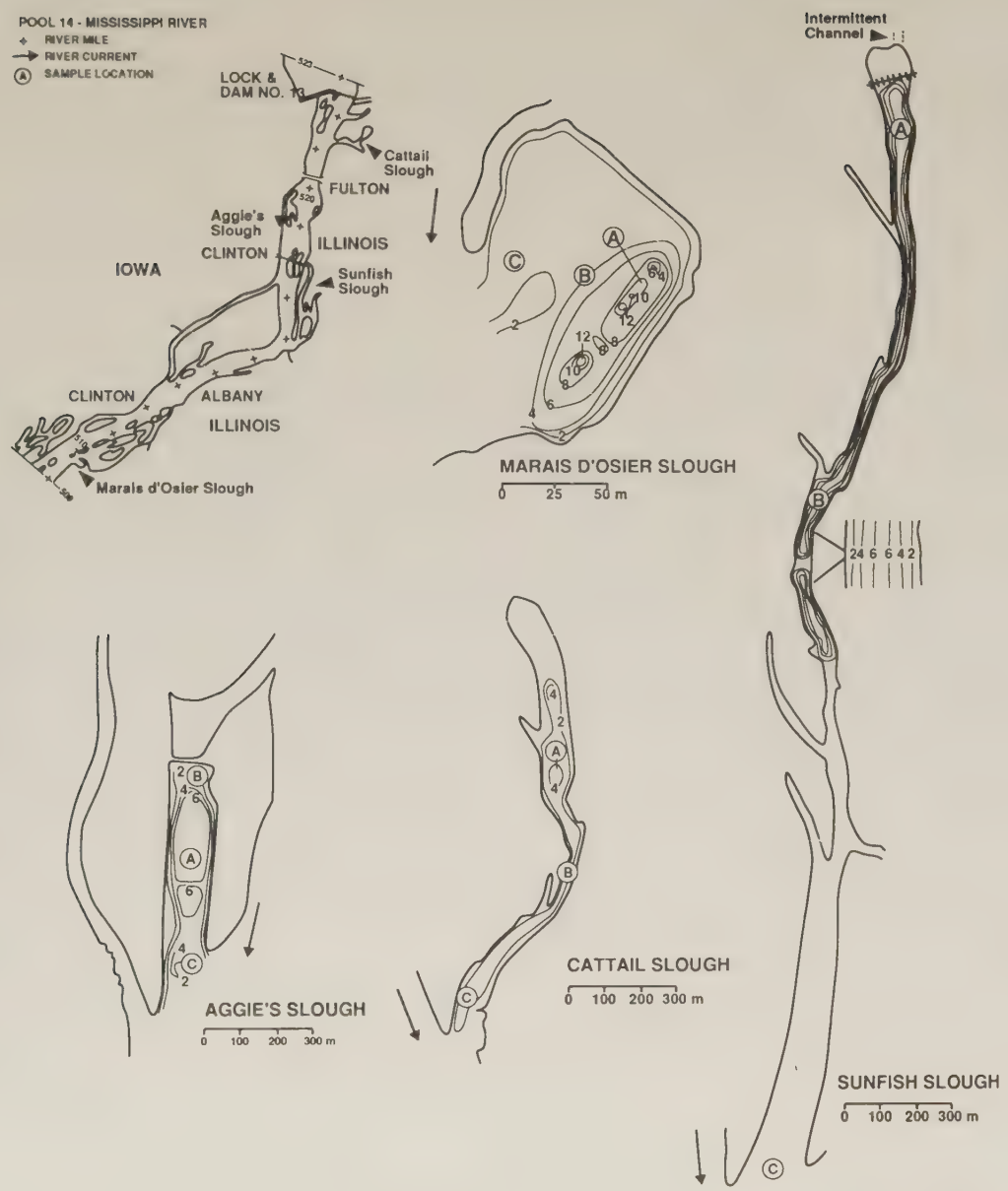

FIG. 1. Location and morphometry of backwater study sites in Pool 14, with 2-m contours indicated on individual site maps.

between pools? The second objective was to determine the factor affecting the health of the fish resulting in incapacitation. Field studies were conducted during the winters of 1983–84 and 1984–85, the interpretation of which was aided by some earlier laboratory studies reported here for the first time.

Study Sites

Our study sites were located on Pool 14 located at 42°N latitude forming the border between Iowa and Illinois. Mississippi River navigation pools, including Pool 14, are broadly characterized as having the upper ends relatively unmodified from the original river with deep sloughs and wooded islands, the midsections with large open areas of marsh habitat, and the lower ends with deeper, open water markedly affected by siltation (Sternberg 1971; Rasmussen 1979). Pool 14, with a surface area of 4165 ha, comprises the section of the river from Lock and Dam 13 at RM 522.5 to Lock and Dam 14 at RM 493.3 (Fig. 1).

Most sampling took place in tailwaters and backwaters. Tailwaters are defined for fisheries purposes as the region of the main channel, the main channel borders, and water directly below a navigation dam to 0.8 km down river characterized by turbulence (Sternberg 1971). Backwaters, composed of sloughs and river lakes and ponds in this study, are characterized primarily by lack of current (Sternberg 1971). Two slough sites, Marais d'Osier Slough and Sunfish Slough, were examined in 1984 and 1985. Cattail Slough and Aggie's Slough were added in 1985. We selected the deepest available slough sites in Pool 14, assuming that differences in environmental conditions, especially temperature, between the two types of habitat were most likely to be detected there. Substrate in the sloughs ranged from hard clay to loose silt and gravel. Human activities (e.g. dredging and gravel quarrying) have resulted in greater depths

relative to many other sloughs in Pool 14 which have been severely aggraded by sedimentation in recent years (McHenry et al. 1984).

Materials and Methods

Drift of Freshwater Drum in Tailwaters

We sampled tailwater areas of Lock and Dam 13 and Lock and Dam 14 in 1984 and 1985 to examine the main channel for drifting freshwater drum during late winter. Tailwaters were selected because the area of this habitat adjacent to the lock facilities is outside the line of navigation, so sampling is not disrupted by boat traffic. Also, the river is constricted at this point, and the increased current enabled a greater proportion of the river volume to be sampled in a given period. Flow with its associated drift from upstream lotic habitats converges at this location.

On 21–23 February 1984, six nets consisting of a 1-m-diameter hoop with a 6-m conical bag of 0.6-cm bar mesh were anchored on a transect perpendicular to river flow approximately 150–200 m downstream from each dam (Fig. 2, for example). Ice floes were present in the channel. Nets were set for 24 and 48 h in the Pool 13 tailwater and for 24 h in the Pool 14 tailwater. Freshwater drum in the catch were counted and total length (TL) was measured. Current was measured near each net in place at 0, 24, and 48 h by a direct-read velocity meter lowered to 0.5 m above the bottom at the estimated position of the net. Flow through each net was calculated by multiplying net cross-sectional area by velocity. Because estimated sample volumes ranged from 221 000 to 962 000 m^3, catch was adjusted to number per 100 000 m^3 of water.

In late winter of 1985, this sampling scheme was repeated over the 1984 transect in the Pool 13 tailwater, where conditions were less hazardous at high water levels. To reduce clogging by debris and increase sampling effectiveness, we changed net design to a 1.13-m-diameter hoop with a 4-m cylindrical bag of 1.0-cm bar mesh. Between 16 and 20 nets were used depending on river conditions at 22 net locations (Fig. 2). Samples were collected 6–12 and 21–27 March 1985 at 24-h intervals. Ice breakup on Pool 13 began during the first sample period. Statistical decisions were made using an alpha of 0.05.

Fish Collections and Observations in Sloughs

In 1984, fish were sampled 20–21 March by deploying 1.8 × 0.9 m rectangular frame trap nets with 13-mm bar mesh and a single 15-m lead on the bottom under the ice. Four nets were set in Marais d'Osier Slough and one in Sunfish Slough for 1 d. Trapnet sampling was repeated in all four sloughs 21–26 March 1985 with two or three nets at each site set for 4 or 5 d. Captured fish were identified and measured.

Further observations of fish in the backwater sites were made in 1985 with SCUBA. On 5 and 6 February the deepest locations at Marais d'Osier Slough and Sunfish Slough were examined by a diver. Dives were performed at these sites again on 21 February, and the deepest area of Cattail Slough was examined on 22 February. Lack of visibility prevented examination of Aggie's Slough. The diver spent 60–75-min examining the bottom within a 45-m radius of the entry hole. A 200 000-candlepower hand-held light was used for illumination. Species composition, size composition, and numbers of fish were estimated and behavior was observed.

State of Health

Physical characteristics of incapacitated freshwater drum were compared with healthy individuals to determine if differences were present that could account for the apparent difference in the state of health. Incapacitated fish, characterized by their inability to escape impingement by the slight current at the barrier net, were collected near the intake of QCS in March 1981. Fish that appeared active and healthy were collected by seining in the Marais d'Osier Slough site during this period.

All fish were frozen and transported to the laboratory. After thawing, individual lengths and weights were recorded and fish were necropsied. Seven characters were examined: (1) fluid in the body cavity, (2) gall bladder distension, (3) bile color, (4) muscle texture, (5) liver color, (6) liver weight, and (7) presence of material in the gut. Body condition factor (K) was calculated by dividing weight (grams) by the cube of length (TL, in millimetres) and multiplying by 100 000. The liver somatic index (LSI) was calculated by dividing the wet weight of the liver (grams) by the body weight (grams) and multiplying by 100. Gonad weight was not excluded from body weight, since all fish were immature. Statistical comparisons between groups were made using an alpha of 0.05.

As an index of nutritional status, total lipids were measured in a sample from each collection. Carcasses were dried to a constant weight at 60°C in a vacuum of −80 kPa and weighed to determine moisture content. Dried carcasses were individually ground through a number 40 screen in a Wiley shearing mill. A sample between 2 and 4 g was weighed to the nearest 0.0001 g. The sample was refluxed for 4 h in diethyl ether in a Goldfisch fat extractor. The solvent was then evaporated from the tared container, and the quantity of lipids present was determined by subtraction.

Physical and Chemical Measurements

In 1984 we measured temperature and dissolved oxygen on 18–19 January, 8–9 February, 6 March, and 21–22 March in Marais d'Osier Slough and in an adjacent side channel where there was a pronounced current. Sunfish Slough was examined 9 February and 6 March. Vertical changes in water temperature were recorded at 2-m intervals from surface to bottom to the nearest 0.05°C during the first three sampling periods. Dissolved oxygen was measured 0.5 m above the bottom with a polarographic probe attached to a 1.9-cm-diameter perforated plastic pipe used to contain the thermistor.

In 1985, sampling procedures were modified so that measurements were repeated at three locations in each slough. Temperature and dissolved oxygen were measured at surface, mid-depth, and near the bottom at the point of maximum depth near the slough confluence with the channel, midslough, and distal to the confluence and also in the main channel border down river of Marais d'Osier Slough at RM 508. Two other backwaters, Cattail Slough and Aggie's Slough, were added to the study, and the four backwaters and the main channel border were examined on 12 and 28 December, 16 January, 5–7 and 20–22 February, and 9–11 and 25–27 March.

To characterize the source of heat in the backwater sites, substrate temperatures were also examined in 1985 on each sampling date at the deepest sample location in each site. A 1.9-cm-diameter plastic pipe fitted with a metal point was pushed into the substrate. Four thermistors flush with the pipe surface and exposed to the outside at the bottom and 30, 60,

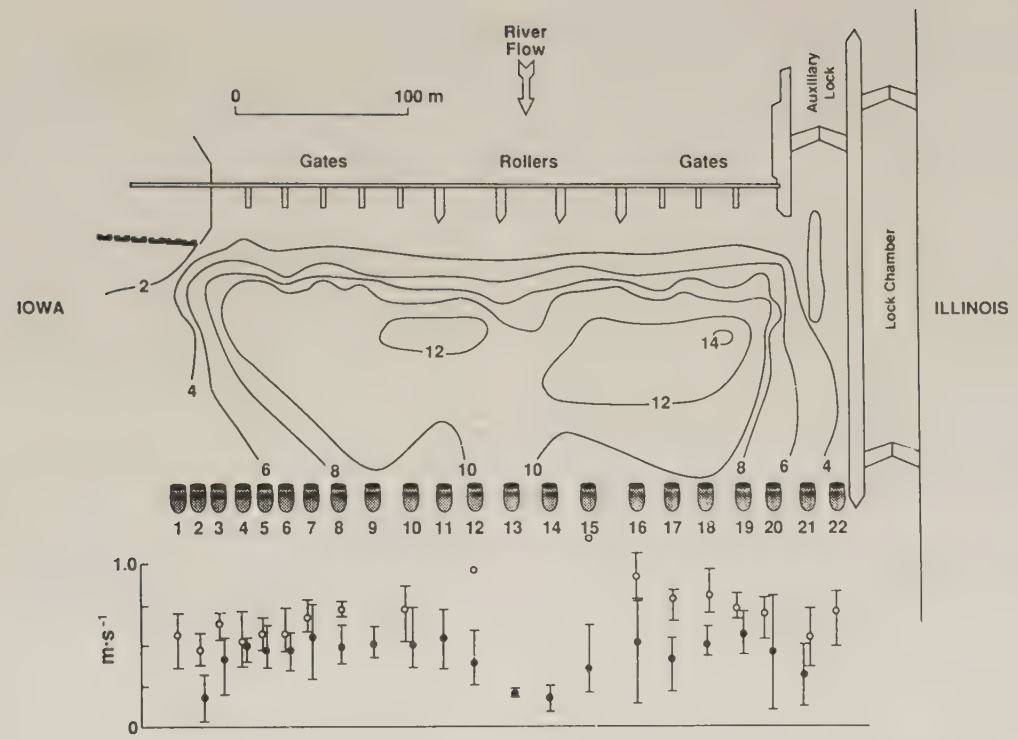

FIG. 2. River morphometry and drift net locations in the Pool 13 tailwater in 1985. Contours are 2-m intervals at normal pool. Mean water velocity and range are indicated for each net location (solid circles: 6–12 March; open circles: 21–27 March).

and 90 cm deeper were used simultaneously to measure substrate temperatures 5 min after insertion.

To examine other possible causes of debilitation in freshwater drum, we used a nonmetallic Kemmerer bottle to collect two water samples 0.5 m above the bottom at the deepest sample location in the sloughs and middepth at the main channel border. One sample was preserved for analysis of total concentrations of copper, iron, and zinc, as indicators of changes in metal species concentrations in the water column as winter progressed. Dissolved carbon dioxide, hydrogen sulfide, total ammonia, methyl orange alkalinity, and pH were measured in the other sample. Samples were taken on 12 and 28 December, 16 January, 5–7 and 20–22 February, and 9–11 and 25–27 March. Metal analyses were not conducted on the 12 December sample.

Laboratory Study

To examine the relationship between temperature and state of health of freshwater drum, laboratory studies were conducted in 1982–83. Fish for the study were collected by shoreline seining in Rend Lake, Illinois (38°N, 89°W), during the fall of 1982, when water temperatures were 21–25°C. After transport to the laboratory, water temperature was initially lowered to 15°C for 14 d and then raised to 20°C to allow effective treatment of fungus and facilitate prophylactic measures for other pathogens over a 30-d period. Feed was offered on alternate days until initiation of the experiment. When disease was no longer evident, fish were distributed in the experimental facility, which consisted of six 700-L covered circular tanks each equipped with a 1-hp water chiller, a supply of dechlorinated tap water, and an incandescent 40-W light source. Two tanks were assigned to each of the nominal final temperatures of 1, 5, and 10°C, and fish were randomly stocked with four tanks receiving 39 fish each and two tanks 40 fish each. Water temperature was lowered at a maximum rate of $1°C \cdot d^{-1}$ and photoperiod was adjusted from 12L:12D to 9L:15D during this period. Final temperatures were set and maintained by a thermostatically controlled relay. Tanks set to 10°C were ±1°C 92.6 and 95.1% of the period and ±2°C 98.6 and 96.7% of the period with extremes of 6 and 14°C; tanks set to 5°C were ±1°C 82.7 and 93% of the period and ±2°C 91.3 and 98.3% of the period with extremes of 1 and 9°C; and tanks set to 1°C were ±1°C 85.2 and 92.2% of the period and ±2°C 90.1 and 100% of the period with extremes of 0 and 4°C based on daily temperature readings. Mortality also was recorded daily. After 12 d at the final temperature, a group of fish was removed from each tank to determine osmolality. Three further removals occurred at 45-d intervals. Since a portion of the fish was removed initially and then again at 45-d intervals, overall mortality was assessed for each of the three intervals based on fish remaining at the beginning of the interval.

At the conclusion of each sample period, individual fish were administered a sharp blow to the head and measured. The caudal peduncle was severed, and blood was collected in heparinized hematocrit capillary tubes. Samples were refrigerated at 4°C up to 3 h and then spun for 5 min in a clinical centrifuge. The plasma fraction was separated from the packed cells, and an 8-μL plasma sample was inserted into a Wescor 5100C Vapor Pressure Osmometer (sensitivity ±2 mosm ($=2$ mmol·kg^{-1})) with at least one replicate per fish.

To compare plasma osmolality of fish held at the three temperatures in the laboratory with both healthy and incapacitated fish from the river, blood samples were taken from seven fish collected by 24-h trapnet sets in the Marais d'Osier Slough backwater site on 21 March 1984 and from eight fish collected

TABLE 1. Daily discharges and catch of freshwater drum by drift nets in late winter 1984 and 1985.

Tailwater	Date	Catch	Sample volume ($m^3 \times 10^5$)[a]	Freshwater drum·$m^{-3} \times 10^5$	River discharge ($m^3 \times 10^5$)[b]
			February 1984		
Lock and Dam 13	21–23	5	2.26	2.20	998
	22–23	2	2.48	0.80	1675
Lock and Dam 14	21–22	5	3.50	1.42	2037
			March 1985		
Lock and Dam 13	6–7	2	3.98	0.50	1598
	7–8	4	7.38	0.54	1675
	8–9	24	6.37	3.76	1861
	9–10	23	6.26	3.68	1979
	10–11	45	6.86	6.55	2000
	11–12	5	6.52	0.77	1998
Lock and Dam 13	21–22	3	6.04	0.50	2091
	22–23	20	5.94	3.37	2086
	23–24	12	6.79	1.77	2159
	24–25	19	7.63	2.49	2197
	25–26	14	9.36	1.50	2224
	26–27	11	9.62	1.14	2297

[a]Estimated from flow measurements at each net at the beginning and end of each sample period.
[b]Data provided by the U.S. Army Corps of Engineers, Rock Island District.

from a 2-h set of the power station intake barrier net on 24 February 1984. All fish were transported to the power station environmental laboratory, held for 4 h while warming to 7°C, individually netted, administered a sharp blow to the head, and measured. The caudal peduncle was severed and two heparinized capillary tubes of blood were collected from the caudal artery. Blood samples were treated the same as those from the laboratory-held fish with at least one replicate per fish. Statistical decisions comparing plasma osmolality between groups were made using an alpha of 0.05.

Results

Drift of Freshwater Drum in Tailwaters

Sampling in the Pool 13 tailwater for 2 d in 1984 yielded seven freshwater drum (Table 1); the Pool 14 tailwater yielded five freshwater drum in the 1-d sample. The daily catches adjusted for flow were 2.20 and 0.80 freshwater drum·100 000 m^{-3} in the Pool 13 tailwater and 1.42 freshwater drum·100 000 m^{-3} in the Pool 14 tailwater. Catch in the Pool 13 tailwater may have been reduced in the second day of sampling by the entanglement of the two nets in the center of the river. This problem was not encountered in subsequent samples.

Two of the captured freshwater drum were dead and decaying. Cause of death could not be determined. The fresh fish were 89–147 mm TL with a mean of 126 mm. The two decaying fish had no caudal fins, and total lengths were estimated to be 104 and 230 mm.

Samples from the drift in the Pool 13 tailwater after 12 d in late winter of 1985 totaled 182 freshwater drum. Effort from 6 to 12 March was 97 net-days yielding 103 freshwater drum, and effort from 21 to 27 March was 100 net-days with a catch of 79 freshwater drum. Effort at a particular net location within

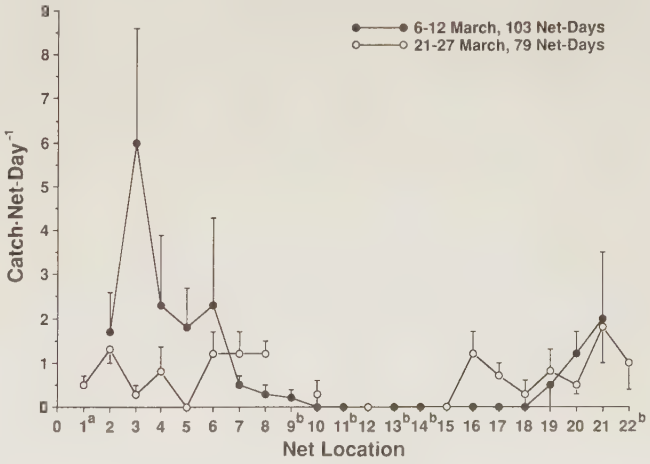
6-12 March, 103 Net-Days
21-27 March, 79 Net-Days
Catch·Net·Day⁻¹
Net Location
0 1[a] 2 3 4 5 6 7 8 9[b] 10 11[b] 12 13[b] 14[b] 15 16 17 18 19 20 21 22[b]

FIG. 3. Catch of freshwater drum by net location in the Pool 13 tailwater during each sample period in 1985. Vertical bars represent either plus or minus 1 SE as space allowed.

a sampling period varied from 2 to 6 d. Daily catch per 100 000 m^3 ranged from 0.50 to 6.55 for 6–12 March and 0.50 to 3.37 for 21–27 March (Table 1). Distribution across the channel was nonuniform during the 6–12 March period (Fig. 3); the daily catch of 1.13 freshwater drum per net-day at the 11 lateral net positions (six on Iowa side and five on Illinois side) was significantly greater than the 0.13 fish·net-day^{-1} at the 11 central net positions (Mann–Whitney test, $P = 0.007$). No significant difference in distribution was detected during the 21–27 March period when the daily catch per net-day was 0.78 in the lateral nets and 0.64 in the central nets (Mann–Whitney test, $P = 0.68$). Daily catch per net-day was not correlated with river discharge (Spearman's rho, 6–12 March: $P = 0.59$, 21–27 March: $P = 0.70$).

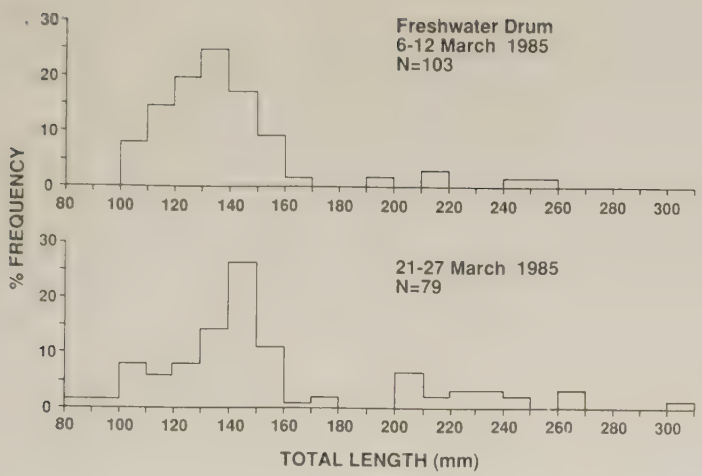

FIG. 4. Length frequency distributions of freshwater drum collected in the Pool 13 tailwater in 1985.

Nearly all (98%) of the freshwater drum in 1985 were live or had only recently died versus being in an advanced state of decay. Small fungal growths were noted on the pectoral or pelvic fins in 33% of the fresh fish. For 6–12 March the modal length group was 130–139 mm, with 90% of the fish less than 160 mm (Fig. 4, top panel). For 21–27 March the mode had increased slightly to 140–149 mm, and 75% of the fish were less than 160 mm (Fig. 4, bottom panel). A second smaller group (19%) was 200–270 mm long. The length distributions of fish from both periods indicated that small fish, of the sizes characteristic of the first or second year of life, were most susceptible to factors causing incapacitation. Their presence in both tailwaters also indicated that drifting fish were not confined to Pool 14 and that the causative agent was not specific to this pool.

Fish Collection and Observations in Sloughs

In 1984, the four nets set for 1 d in Marais d'Osier Slough caught 162 freshwater drum ranging from 95 to 202 mm TL with the mode at 120–129 mm (Fig. 5, top panel). Temperatures in the vicinity of the nets ranged from 0.55 to 1.40°C and dissolved oxygen from 8.2 to 12.8 mg·L^{-1}. The single net in Sunfish Slough contained no freshwater drum after 1 d (3.85°C; 1.9 mg dissolved oxygen·L^{-1}). In 1985, trapnet effort ranged from 8 to 15 net-days at each site, and adjusted catches increased from 2.3 freshwater drum·net-day^{-1} in Sunfish Slough to 5.0 in Aggie's Slough, 43.8 in Cattail Slough, and 102.8 in Marais d'Osier Slough.

Length frequency distributions were unimodal, with most fish 80–170 mm TL, except in Cattail Slough, where the distribution was distinctly bimodal. There the group of large fish ranged from 180 to 260 mm with a second mode at 210–219 mm. Primary modal length groups were 140–149 mm (Marais d'Osier Slough) 120–129 mm (Sunfish Slough), 130–139 mm (Aggie's Slough), and 140–149 (Cattail Slough) (Fig. 5, bottom four panels). Length frequency distributions from trapnet capture in the backwaters were very similar to the stationary net capture in the main channel tailwaters (see Fig. 4).

On 5 February at the Marais d'Osier Slough site an estimated 75 freshwater drum from 50 to 100 mm TL were observed resting on the bottom in shallow, circular depressions with a diameter slightly greater than the length of the fish. Visibility was

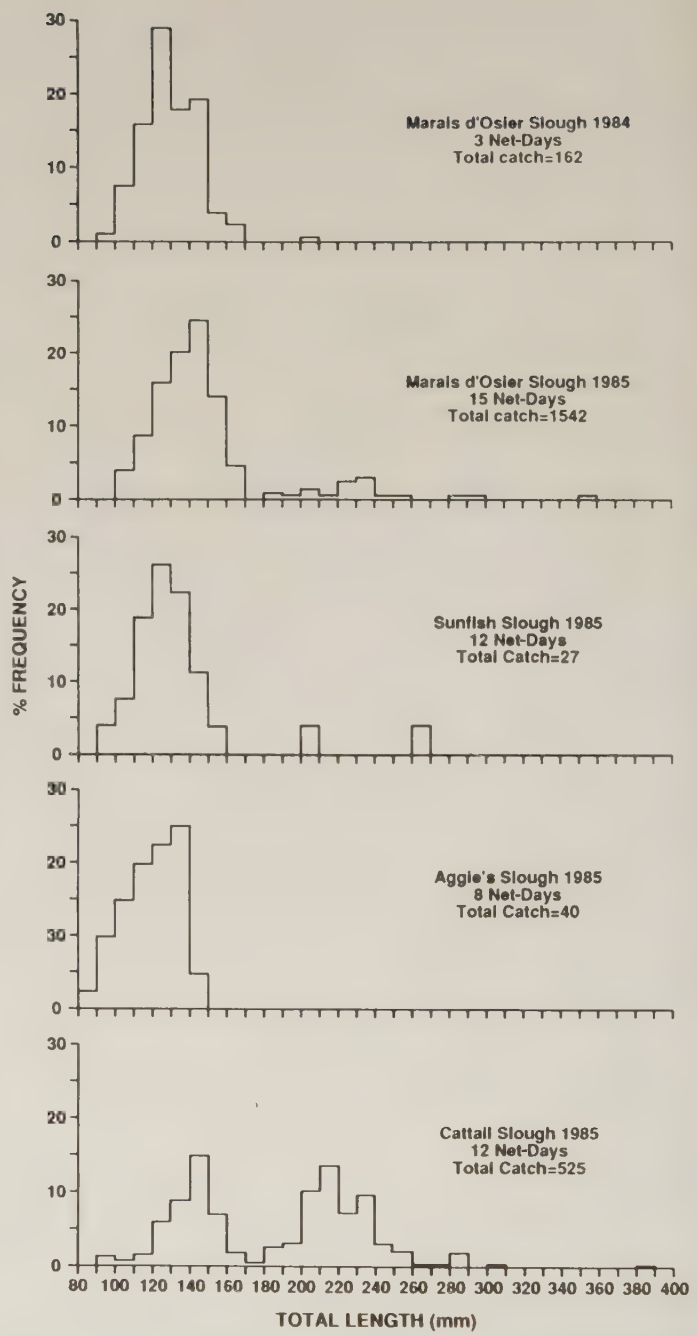

FIG. 5. Length frequency distributions of freshwater drum captured by trap nets in the backwater sites in March of 1984 and 1985.

less than 1 m. These fish allowed the approach of the diver and light within 25 cm. Larger freshwater drum estimated to be 150–200 mm TL were seen swimming 15–30 cm above the bottom and appeared to avoid the diver. In Sunfish Slough, two freshwater drum in the 50–100 m TL range were found on the bottom where visibility was 2 m. On 20 February in Marais d'Osier Slough, 35 larger freshwater drum (TL > 150 mm) were seen swimming, and 10 in the 50–100 mm length group were found on the bottom. Fish activity was greater than on the previous dive. In Sunfish Slough, visibility had increased to 6 m, but no live fish were seen. Dead fish, including 20–30 freshwater drum ranging from 70 to 250 mm, were found on the bottom. In Cattail Slough, three freshwater drum, all less than 150 mm TL, were seen swimming near the bottom, and

TABLE 2. Mean and range of size, condition factor (K_{TL}), and liver somatic index (LSI) of freshwater drum impinged on the intake barrier net at QCS and seined from a Marais d'Osier Slough backwater in March 1981. No significant differences were detected between collections (Student's t-test, $P < 0.05$).

	Barrier net		Seine haul	
	N	Mean (range)	N	Mean (range)
Total length (mm)	113	129 (94–264)	63	136 (90–345)
Body weight (g)	113	27 (7–243)	63	50 (6–698)
K_{TL}	113	1.02 (0.62–1.45)	63	1.00 (0.75–1.70)
Liver weight (g)	90	0.28 (0.04–1.65)	54	0.22 (0.05–0.84)
LSI	90	1.05 (0.34–1.85)	54	1.16 (0.48–2.22)

three dead ones were found on the bottom. Turbidity in Aggie's Slough prevented visual observations.

The presence of large numbers of apparently healthy small freshwater drum in the backwaters, while similar-sized incapacitated fish were drifting in the main channel, indicates that the factor causing incapacitation was not present in backwater habitat. Further, the sedentary behavior of small fish in the backwaters in midwinter suggests that shelter from lotic habitat is required during winter.

State of Health

Based on subjective assessment of appearance, gross indications of starvation including gall bladder distention, clear bile, and watery muscles were not present in fish from the backwater or from the barrier net. Liver color and texture were similar in both groups. Ingested material was not present in any stomachs, but highly digested matter was found in the posterior segment of the intestine in 20% of the barrier net sample and 25% of the backwater sample. Fluid in the body cavity, based on observable flow from a midline incision when the fish was held upright, occurred in 36% of the barrier net fish and 57% of the backwater fish. Mean condition factor was slightly higher in the barrier net sample, but the difference was not significant (Table 2). LSI was higher in the backwater sample, but this difference also was not significant. Lipid content was similar between the two samples for all size groups (Table 3).

One nematode parasite was commonly noted, and it was present in fish from the backwaters and from the barrier net. The nematode, which was only found in the oculo-orbital sockets of the fish and ranged in size up to 150 mm, was identified as a member of the genus *Philometra*. The presence of nematodes was outwardly indicated by a dark red to black ring around the margin of the eye, and the eye was exopthalmic. In the March 1981 samples the parasite was present in 58.6% of the fish from the backwater and 60.0% of those from the barrier net. No other parasites or diseases were evident. The lack of distinction between collections indicates that the cause of incapacitation cannot be attributed to a prior poor state of health.

Physical and Chemical Measurements

Temperature increased with depth in the sloughs. In the deepest area of Marais d'Osier Slough in January 1984, temperature near the bottom was 3°C, in contrast with the 0°C temperature throughout the side channel. Similar results were obtained during the February and March samples, following a general trend of increase in temperature with an increase in depth at any given sample point. Maximum temperature on 8

TABLE 3. Mean percent body fat by 10-mm length group of freshwater drum collected from the barrier net at QCS and from the gravel quarry in Marais d'Osier Slough in March 1981.

Length group (mm)	Barrier net		Gravel quarry	
	N	% body fat	N	% body fat
70–79	2	5.10	7	4.73
80–89	27	5.41	14	4.91
90–99	26	5.75	9	5.02
100–109	32	5.66	18	7.81
110–119	21	6.12	10	5.61
120–129	6	4.93	2	5.83

February was 5.20°C and on 6 March was 2.70°C, again at times when the side channel we measured was at 0.00°C. Minimum dissolved oxygen concentrations were 8.2, 4.1, and 2.1 $mg \cdot L^{-1}$ for each of these three sample periods, and these concentrations occurred at points that corresponded to maximum temperatures. Elevated temperatures were also found in Sunfish Slough in 1984, increasing with depth and distance from the confluence with the river to a maximum of 4.20°C at 5.0 m on 9 February; instrument limitations prevented bottom temperature measurement (8 m depth). Dissolved oxygen was very low throughout the water column, ranging from 1.4 down to 0.5 $mg \cdot L^{-1}$ at the point of maximum depth. On 6 March, maximum water temperature had fallen to 1.00°C, while dissolved oxygen had increased to 12.8 $mg \cdot L^{-1}$ at the same point. In the period between samples the pool stage measured at the Lock and Dam 13 tailwater had risen from 1.95 m to a peak of 3.71 m on 2 March, falling to 3.23 m on 6 March. This rise caused water to flow from the channel into the slough through an intermittent connection at the upper end.

Water temperature profiles at the four backwater sites examined during the winter of 1984–85 were similar to profiles of the previous winter. At all four sites, water temperature was highest near the bottom (Fig. 6). Maximum temperatures under complete ice cover were 2.65°C at the Marais d'Osier Slough site, 4.60°C at the Sunfish Slough site, 1.40°C at the Aggie's Slough site, and 3.15°C at the Cattail Slough site during periods when the main channel was at or near 0°C.

In 1984–85, dissolved oxygen decreased at all sites to minimum concentrations on 5 and 20–21 February, in contrast with the continually high levels at the main channel site (Fig. 6). The lowest concentrations of 1.2 $mg \cdot L^{-1}$ occurred in Sunfish Slough, the site of the extreme low concentration of 0.5 $mg \cdot L^{-1}$ the previous winter. Vertical profiles showed declining dissolved oxygen concentrations from surface to bottom, while ice cover was intact, except in sunfish Slough where concentrations were uniform throughout the water column. Warm rainy weather on 21 February 1985 caused holes and cracks in the ice cover and could have altered the physical/chemical profile so that it differed from sites examined the previous day.

Hydrosoil temperatures increased with depth into the substrate at all four backwater sites (Fig. 7). Maximum temperatures occurred 90 cm into the substrate at all sites and ranged from 3.75°C in Cattail Slough on 11 March to 6.25°C in Aggie's Slough on 28 December. Minimum substrate temperatures at each site were recorded on the bottom and ranged from 0.65°C in Aggie's Slough on 16 January to 2.10°C in Sunfish Slough on 11 March. The greatest difference in temperature over the 90-cm distance was 2.55°C in Marais d'Osier Slough and

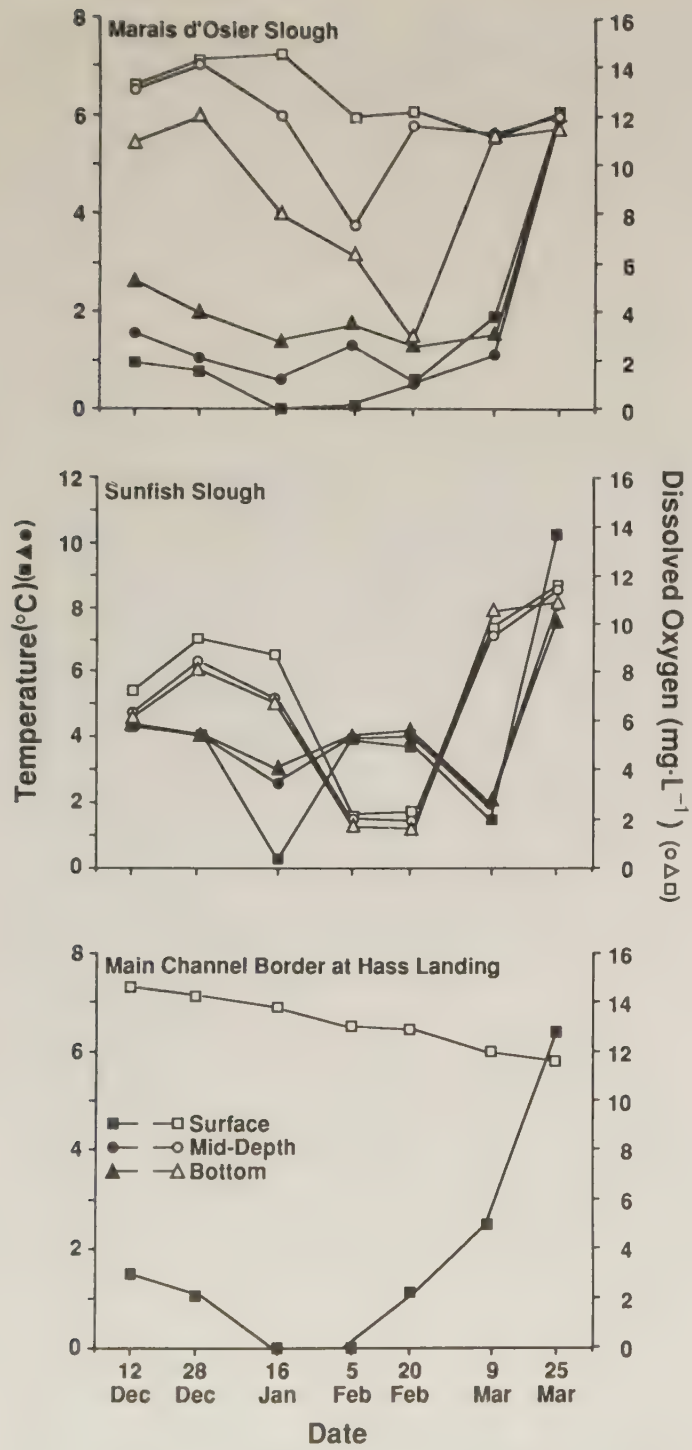

FIG. 6. Temperature and dissolved oxygen profiles at selected backwater and main channel border sites during 1984–85.

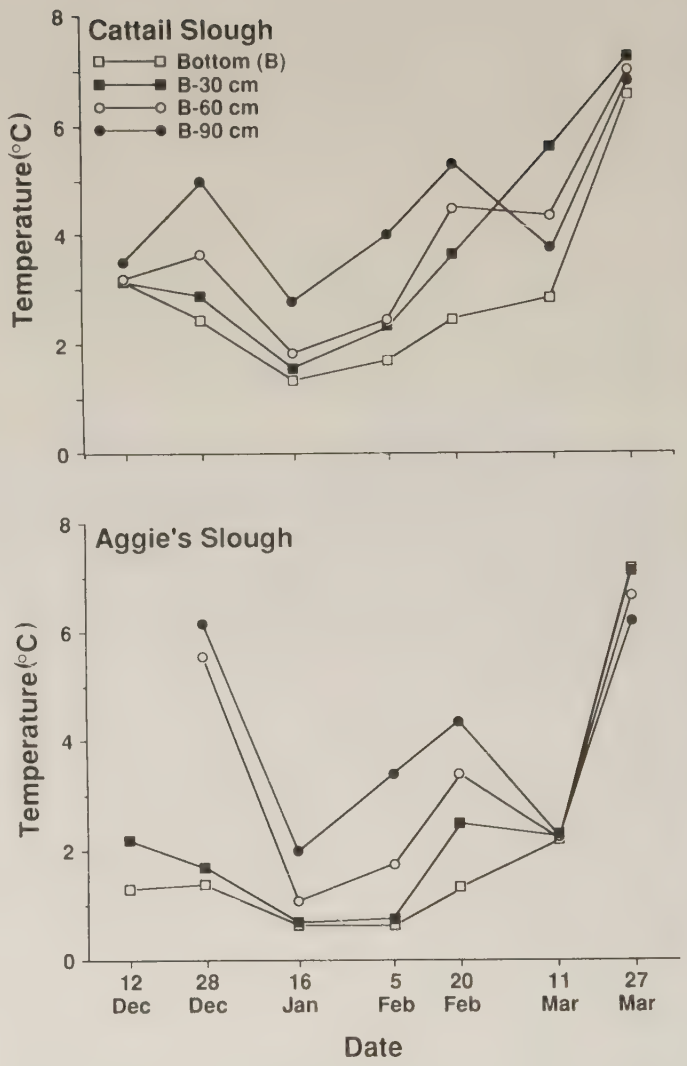

FIG. 7. Substrate temperature profiles of selected backwater sites during 1984–85.

2.85°C in Cattail Slough on 20 February, 2.90°C in Sunfish Slough on 11 March, and 4.75°C in Aggie's Slough on 28 December. Neither absolute temperature nor temperature differences between strata were related to water depth. Substrate temperatures were also independent of substrate composition and compaction.

The highest unionized ammonia (NH_3-N) concentration was calculated to be 0.004 mg·L^{-1}. Dissolved carbon dioxide ranged from 10 to 48 mg·L^{-1}, and methyl orange alkalinity had extremes of 88 and 208 mg·L^{-1}. The pH varied from 6.80 to 7.95. Hydrogen sulfide was not detected in any of the samples. The highest total concentrations of copper, iron, and zinc in the backwaters were 0.068, 3.53, and 0.161 mg·L^{-1}, respectively, with similar concentrations in the main channel.

The most obvious differences between the lotic channel habitat and the lentic backwater habitat were in temperature and dissolved oxygen. Warmer temperatures were available to fish in the backwaters, but unlike channel habitat, some backwaters were susceptible to dissolved oxygen depletion in midwinter, compromising their suitability as winter refuges.

Laboratory Studies

Mortality at 1°C was 56, 29, and 83% during the 0- to 45-d, 46- to 90-d, and 91- to 135-d periods; mortality at 5°C was 41, 33, and 29% during the three successive periods; and mortality at 10°C was 33, 29, and 43% for the three periods. Mortality was similar at all three temperatures during the 46- to 90-d period, but it was highest at the 1°C during the 0- to 45-d and 91- to 135-d periods. In addition, two malfunctioning thermostat relays yielded important information regarding behavior at low temperature. One of the 1°C tanks continued cooling within a 24-h period to 0°C. Fish in this tank demon-

TABLE 4. Mean plasma osmolality and size of freshwater drum held at low temperatures in the laboratory and collected from Pool 14 during winter. Significant differences ($P < 0.05$) indicated by different letters.

	Laboratory tanks											
	1°C			5°C			10°C					
	N	mosm (1 SE)	TL	*N*	mosm (1 SE)	TL	*N*	mosm (1 SE)	TL		Pool 14	
											N	mosm (1 SE)	TL
0 d	11	270 (3.1)	165	13	255 (3.8)	164	16	267 (5.3)	173	Backwater	7	276 (4.3)[a]	133
45 d	7	275 (3.3)	178	9	273 (4.0)	173	7	269 (6.3)	181	Barrier net	8	251 (1.7)[b]	138
90 d	4	265 (2.8)	185	7	272 (6.1)	180	8	267 (4.3)	182				
135 d	1	249 (–)	200	5	259 (4.3)	195	4	257 (2.7)	203				

strated loss of equilibrium and orientation; they swam very slowly, sometimes inverted, and collided with the tank surfaces, or they lay on the bottom. These behavioral abnormalities were also observed in a nominally 5°C tank when the temperature dropped to at least 2°C, although the minimum temperature is not known. Fish in both tanks recovered when the intended temperatures were restored. The focus of the laboratory studies was to determine the effects of long-term exposure to low temperature on fish health, and it was apparent that the 1°C temperature alone was a major stressor as indicated by the higher overall mortality. Also, immediate adverse effects on health were observed when these fish were cooled to near 0°C.

Plasma osmolalities were not significantly different between acclimation temperatures (ANOVA, $P = 0.99$), nor was the interaction of sampling period and acclimation temperature significant (ANOVA, $P = 0.26$). The main effect sampling period was significant (ANOVA, $P = 0.03$) with post hoc analyses indicating that the mean plasma osmolality of 272 mosm at 45 d was significantly greater than the mean of 255 mosm at 135 d (Tukey, $P < 0.05$). Significant trends were present in osmolality through time at 1°C ($P = 0.036$) and 5°C ($P = 0.003$) but not at 10°C ($P = 0.52$) using a second-degree polynomial regression model. Inspection of the data indicates that osmolalities initially rose at all three temperatures and then progressively declined in the last two sampling periods (Table 4). Also, fish at 1°C had the lowest osmolality in those two sample periods. Mean plasma osmolality of freshwater drum collected from the barrier net was significantly lower than that of fish from the backwater (ANOVA, $P = 0.0001$) and most closely corresponded to that of the single remaining fish sampled at 135 d from the 1°C treatment in the laboratory. As a measure of fish health, plasma osmolality further supported the results derived from examination of mortality and behavior of freshwater drum at temperatures from 0 to 1°C in implicating temperature as the factor causing incapacitation in the river.

Discussion

Our study supports the hypothesis that small freshwater drum require refuge from the main channel to survive through the winter. We found that small freshwater drum were incapacitated and drifted downriver in the main channel, while healthy and active fish were present in the backwaters. Thermal regimes differed between backwater and main channel habitat, with water warmer than the channel temperature of 0°C being available to fish in the backwaters. In the laboratory, incapacitation similar to that observed in the main channel occurred at 0°C, and mortality at 1°C was higher than at 5 and 10°C. Based on these results we concluded that small freshwater drum were intolerant of temperatures near 0°C.

Alternative explanations for the cause of incapacitation that were examined were a poor state of health prior to incapacitation and poor water quality. The absence of differences in condition factors and energy reserves between healthy fish from the backwater and incapacitated fish from the barrier net and the lack of other signs of starvation (Love 1970) indicate that gross nutritional depletion was not causing incapacitation. Although parasitism was evident, pathogenicity of this *Philometra* sp. is unknown and it occurred at a similar rate in healthy and incapacitated fish. Water quality factors other than dissolved oxygen were similar between habitats, and concentrations were at levels considered nontoxic to fish. Winterkill in lakes has been primarily attributed to dissolved oxygen depletion, and ammonia, carbon dioxide, and hydrogen sulfide generally have not been found at toxic levels (Scidmore 1957), and our results support this contention. Concentrations of the three representative metals also were less than those reported to exert toxic effects on fish (see Thurston et al. 1979).

Drift of small freshwater drum in the main channel was documented using passive stationary nets that sifted the flow in the tailwaters of both Pool 13 and Pool 14. We assume that these fish were incapable of sustained and directed swimming efforts and were drifting down river. The harsh environmental conditions in tailwaters including high velocity, high turbulence, and scoured bottom strongly suggest that few species, especially small size classes, would normally inhabit this area of the river. The net design that allowed healthy fish to readily escape and the tendency toward higher catches in the lateral areas of the channel both support the assumption that these fish occurred as drift, since drifting objects tend to be displaced toward shore (Leopold et al. 1964). Their presence both entering and leaving Pool 14 indicates that incapacitation and drift is a winter phenomenon which is not restricted to Pool 14.

We did not assess the magnitude of drift in terms of numbers through the winter, but some indication can be derived from impingement collections at QCS. Since 1984, the mean annual freshwater drum impingement has been 135 000 fish. Age analysis indicates that 87% are age 0 and age 1, with sizes ranging from 80 to 150 mm TL. Impingement occurs during every month of the year, but the largest numbers are encountered from February through April and are often an order of magnitude higher than the summer and fall numbers even though cooling water intake volume during November through mid-April is only half of that during the rest of the year. Examination of winter impingement indicates that up to 90% of these fish are either dead or moribund, with the proportion of dead fish to moribund fish increasing through the winter (L. LaJeone, Quad Cities Nuclear Power Station, Cordova, IL 61242, pers.

comm.). Our collections from the tailwaters and those from the intake screens at QCS were largely composed of small fish in the first year of life, suggesting that this is the most vulnerable group to factors causing incapacitation and that large numbers are exposed to these factors. Evidence of size-dependent low-temperature mortality during winter exists for young-of-the-year of several other species including smallmouth bass (*Micropterus dolomieui*) (Oliver and Holeton 1979; Shuter et al. 1980), largemouth bass (*Micropterus salmoides*) (Toneys and Coble 1979; Isely 1981; Miranda et al. 1984), bluegill (*Lepomis macrochirus*) (Toneys and Coble 1979), sander (*Stizostedion lucioperca*) (Svardson and Molin 1973), common carp (*Cyprinus carpio*) (Svardson and Molin 1973), and cisco (*Coregonus artedii*) (Edsall and Colby 1970).

Aggregations of healthy freshwater drum, corresponding to the same size classes as those occurring as drift, were observed and collected in backwaters. In midwinter the small fish were inactive, resing in shallow depressions on the bottom, but by late winter, activity had increased enough to allow effective use of trap nets. Movement by other fish species to specific wintering sites, often characterized by lack of flow or warmer temperatures, has been documented. McLean et al. (1985) found living threadfin shad (*Dorosoma petenense*) in coves where groundwater seepage resulted in temperatures 3–4°C warmer than the ambient lethal temperature characteristic of the rest of the reservoir. During winter, smallmouth bass congregate in deep, still waters (Henshall 1904; Beeman 1924; Langlois 1935; Hubbs and Bailey 1938; Webster 1954; Munther 1970). Minnows have been found to overwinter 0.5 m into gravel substrates, in logs, and under mats of twigs (Emery 1978). Most fish species collected in the Missouri River main channel during winter are found in "wintering holes" in the vicinity of wing dikes (Hesse and Newcomb 1982). Rock cover and "interspacial" areas of sand provide winter habitat for catfishes (Ictaluridae) in the Mississippi River (Hawkinson 1980). The blacknose dace (*Rhinichthys atratulus*) winters in crevices beneath stream rubble in southern Ontario (Cunjak and Power 1986). A number of typical midwestern reservoir species were found to aggregate in areas of influx of warmer groundwater during winter (Hancock 1954). Even juveniles of coldwater species such as coho salmon (*Oncorhynchus kisutch*) appear to have specific winter habitat requirements in the form of deep pools, log jams, undercut banks with tree roots, and riverine ponds (Bustard and Narver 1975; Peterson 1982a, 1982b; Tschaplinski and Hartman 1983).

Main channel border and side channel habitat fell to temperatures at or near 0°C for much of the winter and were uniformly cold from top to bottom, which is common for lotic environments during subfreezing weather (Parsons 1942; Devik 1944; Pivovarov 1973). Since backwaters exhibit lentic conditions within the riverine system, we expected that the thermal profiles of these areas would be similar to those of ice-covered lacustrine environments (see Welch 1935). This was confirmed in all four backwaters where the water column was thermally stratified under ice cover, and elevated temperatures relative to the main channel persisted through the winter. Bottom temperatures between backwaters did not appear related to morphometric variables, but bottom temperatures within a site increased with depth, similar to lakes where water at maximum density at 4°C flows downslope to pool in local depressions (Mortimer and Mackereth 1958). Although the small, shallow depressions associated with the small freshwater drum in the backwaters may be only incidental to their sedentary behavior during winter, these could also provide a trap for warmer water. Retention of warmer water within a backwater may be dependent on the relation of its morphometry to that of the adjoining channel, i.e. its ability to trap warmer water. The thermal gradient in the substrate of the backwaters also was similar to lakes where heat stored in the substrate during the summer is conducted and reconvected to the water during winter (Birge et al. 1927; Mortimer and Mackereth 1958). The higher temperatures and lack of a distinct vertical profile in Sunfish Slough indicated that another thermal source was present. Ice thickness at this site was 20 cm when it was 45 cm at the other backwaters, and conversations with local residents led us to conclude that groundwater inflow was occurring. Springs have been identified as maintaining higher temperatures in localized areas of rivers and streams during winter (Benson 1953; Sheridan 1961; Schreier et al. 1980), and groundwater inflow would be expected to function similarly in Mississippi River backwaters, as an additional mechanism to provide warmer water for fish seeking thermal refuges.

Exposure of fish in backwaters to the prevailing low temperature of the channel habitat can occur when the river stage increases from ice and snow melt during late winter and inundates some of these areas as observed in Sunfish Slough. When the channel temperature is low enough to cause incapacitation, the resulting current could transport the fish from the backwater to the channel.

Dissolved oxygen concentrations decreased from surface to bottom, another characteristic of lakes under ice (Greenbank 1945). Dissolved oxygen depletion in the backwaters could stimulate fish to move toward the higher concentration in the adjacent flowing channels where they would be exposed to lower temperatures. During winter, several fish species have been observed to orient to and migrate to habitats with higher dissolved oxygen concentrations but lower temperatures as depletion occurred (Magnuson et al. 1985). Largemouth bass and bluegill have demonstrated a threshold avoidance response to low oxygen concentrations in the laboratory (Whitmore et al. 1960). Sunfish Slough has extremely low concentrations in midwinter, possibly attributable to inflow of hypoxic groundwater, and dissolved oxygen depletion is suspected as the cause of the extensive fish kill at that site. Subsequent inundation by channel flow would move these fish into the drift of the main channel.

Mortality in the laboratory study occurred through the 135-d period, indicating that low temperature tolerance of freshwater drum is a function of time as well as temperature. Based on metabolic rate and depletion of energy stores, the highest mortality would be expected at 10°C and the lowest at 1°C in the laboratory treatments. However, the 1°C group had the highest overall mortality, followed by the 10°C group and then the 5°C group, leading us to conclude that the lowest temperature had other adverse effects on fish health. This is further supported by the lower osmolality in fish at 1°C than in those at 5 and 10°C with corresponding differences in fish collected from the main channel near 0°C versus those from a backwater near 3°C.

Osmotic disturbances in other species caused by low temperature exposure include decreased ability to regulate sodium, chloride, and glucose (Bergstrom 1971; Umminger and Bair 1973; Wendt and Saunders 1973; Virtanen and Oikari 1984). Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and brook trout (*Salvelinus fontinalis*) all demonstrated large variations in osmolality near 0°C explained by

reduced osmoregulatory capacity (Saunders et al. 1975; Saunders 1986). To determine actual tolerance to winter thermal conditions, exposure time may have to correspond to the actual duration of environmental temperatures. In addition, small changes in temperature may greatly affect the health of the fish. Fish held at 1°C that were temporarily lowered to 0° became incapacitated but recovered when temperature was restored to 1°C. Doudoroff (1942) described immediate effects, termed primary chill-coma, to lethal low temperatures: "mild distress and disturbance of equilibrium to a violent, convulsive paroxysm." If fish were returned to nonlethal temperatures within a short period, they rapidly recovered with no apparent permanent effects. Secondary chill-coma described the gradual loss of response to stimulus and cessation of opercular movements over periods of hours to days. The persistent mortality of freshwater drum at 1°C through the 135 d in the laboratory appears to correspond to Doudoroff's secondary chill-coma, and the behavior of these fish near 0°C is described by the mild indications of primary chill-coma. The assumption that freshwater fish in regions of persistent winter ice cover are physiologically adapted to withstand water temperatures down to 0°C, i.e. until freezing occurs, may not be valid for all species and size classes. In tolerance tests of seawater-adapted young chum salmon (*Oncorhynchus keta*) and young sockeye salmon (*O. gorbuscha*) to temperatures near 0°C, neither species survived more than 3 d at −0.5°C, at least 0.2°C above their freezing point (Brett and Alderdice 1958). While it cannot be concluded that low temperature is a significant cause of mortality in salmonids, it is evident that the lower lethal limit is not always determined by the freezing point of the body fluids of the fish.

The apparent incongruency between the presence of a thriving commercial fishery for freshwater drum in Pool 14 and the occurrence of large-scale drift and probable mortality of young freshwater drum caused by low temperatures characteristic of Pool 14 can be explained by the ability of young freshwater drum to exploit the thermal differences between riverine habitats. Intolerant of the 0°C temperature in the main channel, these fish can survive in winter thermal refuges. Some of the most prominent habitats that provide warmer conditions are the river backwaters composed of sloughs, lakes, and ponds. However, existing backwaters are disappearing due to sedimentation, while the formation of new ones is mostly precluded by man's constraints on meandering and other fluvial processes that create new backwaters. Implications for freshwater drum and other fish populations which may require winter thermal refuges provided by backwaters are increased susceptibility to winter mortality and increased fluctuations in population size as winter severity becomes a greater factor in determining year-class strength.

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Influence of Lake pH and Macrograzers on the Distribution and Abundance of Nuisance Metaphytic Algae in Ontario, Canada

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France, R. L., and P. M. Welbourn. 1992. Influence of lake pH and macrograzers on the distribution and abundance of nuisance metaphytic algae in Ontario, Canada. Can. J. Fish. Aquat. Sci. 49: 185–195.

Thirty-six softwater lakes in central Ontario were sampled during 1988 to determine the relationship between metaphytic Zygnematacean algae and lake pH and the degree of complementarity between the presence of metaphyton and that of crayfish and tadpoles. A stratified sequential sampling program was developed to quantitatively measure and to objectively categorize average metaphyton abundance for each lake. A significantly greater proportion of lakes below pH 6 were found to contain extensive accumulations of metaphyton compared with lakes above pH 6. Lake pH and alkalinity were significantly correlated with metaphyton abundance. Macrograzer density did not significantly influence the presence or absence of metaphyton, but reduced metaphyton abundance in those lakes which, because of their low pH or shallow depth, were predisposed to its accumulation. Managerial implications regarding the stocking of crayfish or tadpoles to limit the nuisance proliferation of metaphyton, and the importance of predaceous fishes in structuring this interaction, are discussed.

Trente-six lacs à eau douce du centre de l'Ontario ont été échantillonnés en 1988 afin de déterminer le rapport entre les algues zygnématacées du métaphyton et le pH du lac et le degré de complémentarité entre la présence du métaphyton et celle des écrevissces et des têtards. Un programme d'échantillonnage séquentiel stratifié a été élaboré afin de quantifier et de catégoriser de façon objective l'abondance moyenne du métaphyton dans chaque lac. Une proportion significativement plus élevée de lacs à pH inférieur à 6 que de lacs à pH supérieur à 6 abritaient des accumulations de métaphyton importantes. Le pH et l'alcalinité des lacs présentaient une corrélation significative avec l'abondance du métaphyton. La densité de macrobrouteurs n'influait pas de façon significative sur la présence ou l'absence du métaphyton, mais avait pour effet de réduire l'abondance de ce dernier dans les lacs favorables à son accumulation à cause de leur pH peu élevé ou de leur faible profondeur. Les auteurs traitent, dans le contexte de la gestion des lacs, de l'ensemencement par des écrevisses ou des têtards afin de limiter la prolifération nuisible du métaphyton et de l'importance des poissons prédateurs pour la structuration de telles interactions.

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In a recent survey of over 5000 cottagers on recreational lakes in the Parry Sound – Muskoka – Haliburton region of central Ontario, Canada, SPR Assoc. Inc. (1986) concluded that approximately half of the study lakes had "some" development of filamentous algae and that about 16% of these lakes had "considerable" accumulations. Because mass proliferation of filamentous algae (termed "metaphyton") can clog shorelines, the socioeconomic impacts of this problem are immense. These districts comprise one of the premier tourist regions of the province of Ontario (Smith and Mullamoottil 1979; Downing 1986). For those cottagers residing on lakes characterized by metaphyton, the proliferation of algae was ranked higher than either perceived direct effects of acid rain (i.e. diminishing sport fish stocks) or declining water quality (i.e. Secchi disc transparencies) as the chief concern related to overall evaluation of lake problems. Particularly, these metaphyton concerns were perceived to be a reduced enjoyment of swimming and boating activity, reduced minnow populations (ascribed to detrimental effects of filamentous algae on fish spawning), reduced aesthetic appreciation of the lake and shore, and human health concerns. Other issues mentioned by cottagers relating to metaphyton development included adverse effects on tourism and real estate values. The SPR Assoc. Inc. (1986) report concluded that "while detailed field and laboratory studies are currently underway to determine the causes of algal growth, little information exists on the extent of such growth in softwater lakes in central Ontario." This subject was addressed by the present study.

The development of metaphytic Zygnematales algae is recognized to be a characteristic feature of anthropogenically acidifying lakes (Stokes 1984, 1986). The phenomenon is widespread, having been identified in lakes from southern Sweden (Grahn et al. 1984; Lazarek 1980), southern Norway (Hendrey 1982), the Adirondack Mountains of New York (Hendrey and Vertucci 1980) and New Hampshire (Stevenson et al. 1985), in addition to central Ontario (Howell et al. 1990; France et al. 1991; Stokes 1981). Further, the experimental acidification of stream channels (Hendrey 1976; Parent et al. 1986; Planas and Moreau 1986), limnocorrals (Muller 1980), small streams (Hall et al. 1980), and whole lakes (Brezonik et al. 1986; Turner et al. 1987) has also documented increases in the biomass of filamentous green algae following such treatment. Nonexperimental evidence that metaphytic algae increases with

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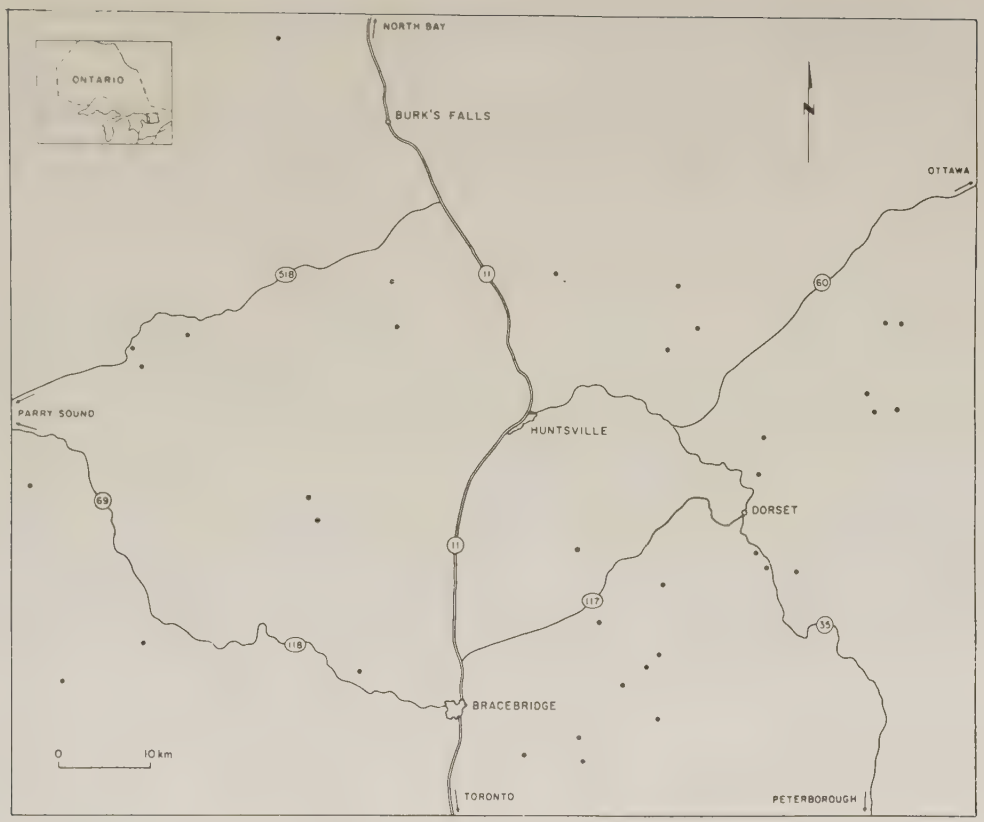

FIG. 1. Location of 36 study lakes in central Ontario.

acidification is, however, mostly "either circumstantial or anecdotal" (Stokes 1981). This statement is still apt. The only published study which suggests that presence of metaphyton is more frequent in acid-sensitive than in nonsensitive lakes is based on the responses of a mail questionnaire survey (SPR Assoc. Inc. 1986). The first objective of the present study was to provide a quantitative assessment of the extent and abundance of metaphytic "clouds" within a series of acid-sensitive (pH 5.3–6.6) Ontario lakes. Given the potential utility of metaphyton as a biomonitor of lake acidification (Stokes and Howell 1988), an ancillary objective was to design a cost-effective and efficient sampling protocol based on appraisal of metaphyton abundance in terms of biovolume/biomass rather than areal cover as is presently used by the Ontario Ministry of the Environment (OMOE) (Jackson et al. 1990).

Several hypotheses have been advanced to explain the "bloom" (sensu Schindler 1980) of filamentous algae in acidifying lakes (Hendrey 1976; Stokes 1981). One of these hypotheses suggests that a diminished grazing pressure through extirpation of herbivores results in less removal of algae in acidic than in neutral waters, thereby promoting an increase in algal standing biomass. In previous research (France et al. 1991), no overt or consistent relationships were found between the abundances of small (cladocerans, oligochaetes, dipterans, etc.) or medium (amphipods, gastropods, emphemeropterans, etc.) sized grazers and the presence or absence of metaphyton. Abundances of macrograzers (crayfish, algivorous fish, and perhaps tadpoles) were, however, reciprocally related to the abundance of metaphytic algae. If alteration in grazing pressure can be implicated as an important factor related to metaphyton occurrence and proliferation, these findings suggested that large grazers would be the most likely candidates. Because only five lakes were sampled, the acceptance of this hypothesis must be tempered (France et al. 1991). Therefore, the present research was designed to provide a broader framework from which to verify or refute the correlative associations observed in the 1987 study. The second objective of the present research was to determine whether a negative relationship exists between metaphyton accumulation and macrograzer abundance. In addition, because lake nutrient status (E. T. Howell, Institute for Environmental Studies, Toronto, Ont., unpubl. data) and morphometry (Howell et al. 1990) may affect development of filamentous algae, we also evaluated lake differences in these variables with respect to metaphyton abundance.

Methods

Study Lakes

The lakes sampled in this survey are located on the Canadian Shield within the Parry Sound – Muskoka – Haliburton districts of central Ontario (Fig. 1; Table 1). These lakes occur within small, glacially formed drainage basins, generally have low productivities, and are ice-covered from December to April. Detailed descriptions of lake geomorphology, chemistry, and biology are available in Dillon et al. (1987a). Selection of study lakes was based on a number of criteria: (1) only lakes which had been classified by the OMOE as being acid sensitivity "1," "2," or "3" (i.e. alkalinities <100 $\mu eq \cdot L^{-1}$) were considered; (2) lakes with perimeters larger than 12.2 km or smaller than 1.9 km were excluded even if known to develop nuisance proliferations of metaphyton; and (3) highly coloured lakes (>90 Hazen units) were also avoided regardless of their metaphyton status. Approximately equal number of lakes were selected (based on a priori knowledge from cottager surveys

TABLE 1. Description of study lakes arranged in order of sampling date for macrograzers. Data for location in degrees latitude/longitude, TP in $mg \cdot m^{-3}$, Secchi depth in m, and for crayfish and tadpoles as average numbers caught per trap.

Lake	Location	Township	pH	TP	Secchi	Crayfish	Tadpoles
Plastic	4512/7846	Sherborne	5.6	5.4	6.6	0	0.23
Crosson	4506/7903	Oakley	5.5	9.9	4.0	1.11	0.18
Grindstone	4511/7853	Rideout	5.3	5.4	5.7	0	0
Tock	4516/7853	McClintock	6.1	5.5	5.6	0.08	0
Shoe	4512/7855	Rideout	6.4	5.7	6.1	0.30	0.70
Heney	4507/7906	McLean	5.9	9.0	4.1	0.88	0.15
Clinto	4519/7852	McClintock	6.2	5.3	6.2	0.23	0.13
Ril	4510/7900	Rideout	6.4	8.2	3.4	0.08	0
Menominee	4512/7909	McLean	6.1	6.7	2.5	0.10	0.13
Little Leach	4502/7901	Oakley	5.3	11.5	2.4	0	0
Wolf	4526/7842	Livingstone	6.0	6.4	3.9	0.03	0.05
Livingstone	4522/7843	Livingstone	6.3	4.3	7.1	0.56	0
Dotty	4528/7900	Finlayson	6.2	7.5	3.5	1.11	0.15
Toad	4526/7856	Finlayson	5.5	7.3	5.1	0	0.23
Leonard	4504/7927	Monck	5.8	4.0	5.8	0	0.03
Pine	4504/7904	Oakley	6.5	4.9	5.1	0	0.98
Spring	4501/7908	Draper	5.5	7.4	5.9	0	0
Prospect	4459/7908	Draper	6.2	9.0	3.6	0.15	0.13
Bear	4520/7824	Livingstone	6.5	3.4	5.8	1.60	0.03
Kimball	4521/7841	Livingstone	6.2	3.3	7.0	1.05	0
Crown	4526/7840	Livingstone	6.2	6.9	6.1	1.60	0.15
Rutter	4515/7931	Cardwell	5.6	6.2	4.5	0	0
High	4514/7930	Cardwell	6.4	5.1	4.4	0.65	0.98
Round	4528/7924	McMurrich	6.4	8.2	3.5	1.06	0.13
Hughes	4542/7934	Chapman	6.3	16.6	2.6	0.03	0.15
Buck	4525/7923	Chaffey	6.0	7.4	2.5	0.20	0
Foote	4529/7910	Sinclair	6.1	7.7	2.5	0.80	0.05
Diamond	4523/7946	Christie	5.9	8.4	3.3	0.20	0.70
Sugar	4522/7946	Christie	5.9	8.0	5.4	0.23	0.23
Manitawaba	4524/7942	Monteith	5.8	5.5	5.7	1.02	0
Salmon	4515/7958	Foley	6.5	7.4	3.8	0.8	0
Gullfeather	4507/7904	Oakley	5.9	7.9	3.4	1.15	0
Bentshoe	4525/7856	Draper	6.0	8.1	4.7	0.10	0
Galla	4504/7952	Freeman	6.4	7.8	4.7	0.30	0
Myers	4506/7945	Freeman	6.3	9.0	3.1	1.25	0
Little Clear	4524/7903	Sinclair	6.6	6.8	5.2	1.40	0.18

(SPR Assoc. Inc. 1986) and/or direct appraisals (M. Jackson, OMOE, unpubl. data; France et al. 1991)) which had either "Nil" ($n = 19$) or "Considerable" ($n = 18$) developments of metaphyton.

Metaphyton Sampling

Sampling effort was stratified by lake size and shape. From detailed bathymetric maps, lakes were divided into approximately equally sized areas based on large-scale shoreline typography (Fig. 2). For lakes with perimeters less than 4 km, there was one division (e.g. Myers Lake), for lakes with perimeters between 4 and 9 km, there were two divisions (e.g. Tock Lake), and for lakes whose perimeters exceeded 9 km, there were three divisions (e.g. Leonard Lake). Such a subdivision of the lakes ensured that sampling would always take place within each particular region (see below), rather than occurring all in one small area which might have happened through a completely randomized design. Because previous research by us had suggested that littoral slope and fetch could influence metaphyton biomass (Howell et al. 1990), it was considered important to balance our present sampling effort in relation to shoreline features. In this respect, the complete lake perimeter was partioned into a series of small zones representative of three strata: "point," "bay," and "straight." Precise sampling locations for one of each of these strata were randomly chosen within each of the large-scale lake divisions (Fig. 2). Such a sampling design was believed to provide an integrated assessment of metaphyton abundance for the entire lake.

The quantitative estimation of metaphyton abundance was based on techniques developed previously by us (Howell et al. 1990; France et al. 1991). At each sampling position, a 0.5 × 0.5 m (bottom area) × 1.0 m (height) box was slowly lowered onto the substrate, effectively isolating a volume of water and any metaphytic algae which occurred there. Metaphyton was removed from the quadrants with a strainer, biovolume measured in a graduated cylinder after the algae had been spun free of most of its water, and the sample then stored in a plastic bag. Samples were later dried at 50°C for 48 h for biomass determinations. At every sampling location, a 15-m transect line, secured at both ends with diving weights, was placed parallel to the shoreline at a depth of about 1 m. Five samples were then taken at random distances along each transect.

Lakes were visited between August 13 and 28 at a time roughly coincident to maximum metaphyton abundance in these lakes (Howell et al. 1990; France et al. 1991). Those lakes

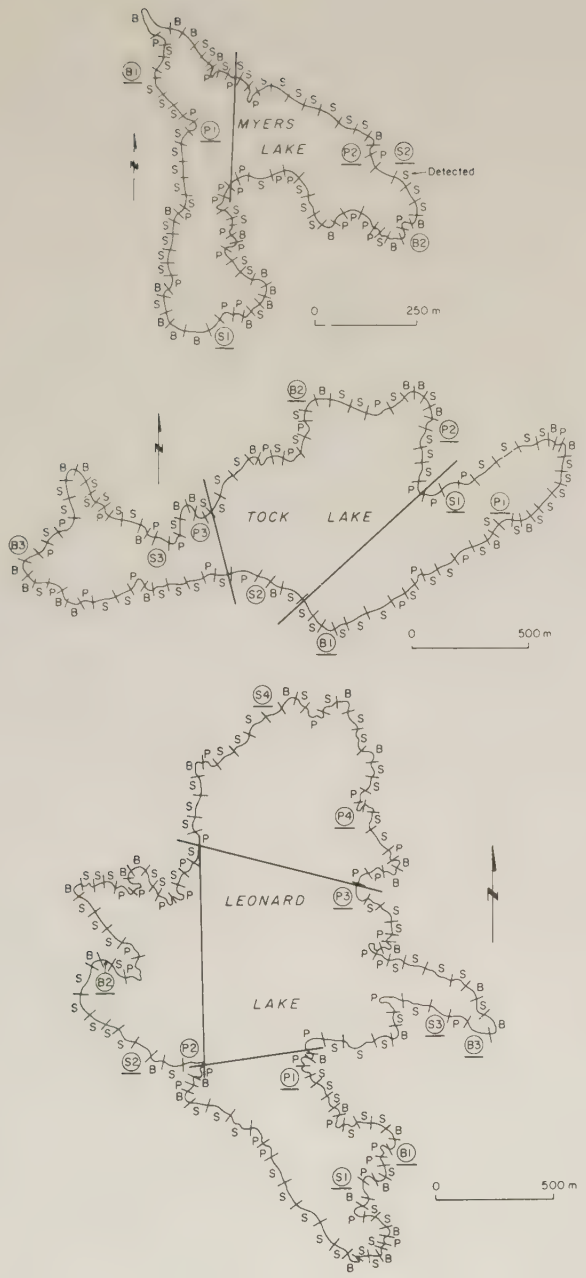

FIG. 2. Stratified sampling design for three representative lakes. S = straight, B = bay, and P = point. Randomly located transect locations are indicated by circles; those that actually had to be surveyed before a decision was made in regard to metaphyton abundance are underlined.

identified as potentially containing metaphyton by the OMOE cottager survey or by us from the previous collection of grazers (see below) were sampled first. By waiting for an additional week before sampling those lakes not believed to contain metaphyton, this allowed for further opportunity for filamentous algae to develop there. Estimation of metaphyton biomass has been previously based on collection of between 80 and 150 samples per lake (Howell et al. 1990; France et al. 1991). Such a labour-intensive effort is, however, unsuitable for synoptic surveys or biomonitoring purposes. One cost-effective sampling procedure with a long-established tradition of use in applied entomological surveys is sequential sampling (e.g. Morris 1954). Recently, Jackson and Resh (1988) and France (1991) have advanced the use of sequential sampling in the design of zoobenthos biomonitoring program.

The great strength of sequential sampling is in reducing the total number of samples needed to categorize a population compared with the sample requirements for fixed sample size procedures. For the latter, all collected samples are examined, statistical parameters estimated, and data then classified based on hypothesis testing. With sequential sampling, decisions relating to a priori specified tests of hypotheses with known error risks are iteratively performed after each sample is examined until the minimum information needed to make a classification is obtained (Jackson and Resh 1988). Data can be interpreted in a graphical format as a pair of decision lines enclosing a band of no-decision from which, with sequential sample assessment, the cumulative plotted points must "escape" before a density classification is satisfactorily defined (Morris 1954; France 1991). Statistical parameters of the threshold classifications are calculated only once from baseline information. The advantage is that no time is wasted in superfluous sampling in situations where metaphyton biovolume falls quickly and distinctly into a particular classification category. As long as the plotted points remain between the decision lines, sampling continues until some predetermined maximum sample number is reached. A related use of sequential sampling is in the combination of several independent tests to sort data into broad categories in which one has considerable confidence in assigning abundances and the resulting correctiveness of any conclusions reached (Green 1979; France 1991). Armitage (1950) provided formulae for a combined sequential test involving three binomial distributions. However, he concluded that such a test was not very different from the method of a series of paired tests such as used in the present study.

Three essential elements must be defined a priori in the creation of a sequential sampling program. First, the classification thresholds or class limits (which describe the decision lines) must be set using available information. Data from Plastic Lake in 1986 (Howell et al. 1990), Plastic, Bentshoe, and Gullfeather lakes in 1987 (France et al. 1991), and Plastic Lake in spring – early summer 1988 (E. T. Howell, unpubl. data) were used for this purpose. Classification thresholds were selected as being greater than $\bar{X}$'s $\pm$ 2SE's to achieve nonoverlapping confidence intervals as a conservative adjustment for the effects of sampling error on the mean (Jackson and Resh 1988). Seven categories were designated: "Nil" (N), "Detected (D) (observed in the lake but never sampled in any of the transects), "Present" (P), "Rare" (R), "Common" (C), "Abundant" (A), and "Very Abundant" (VA). The second step in designing a sequential sampling plan is to establish the mathematical distribution of the data. Metaphyton biovolume was found to conform to a Poisson distribution for the "Rare" and "Present" categories and a negative binomial with a common dispersion index of 0.54 for the other categories. Finally, the probability of classification errors (Types I and II) was set at 0.05 (Jackson and Resh 1988). Together, the classification thresholds, α, β, and k_c, are used to calculate the decision lines (Fig. 3) with the formulae presented in Morris (1954) or Jackson and Resh (1988).

Upon arrival at a lake, sampling was commenced within that particular division area in which the water access was located. After sampling all three shoreline strata (point, bay, straight), sampling was continued in the adjacent division area, again on the designated strata in sequence of their occurrence as we

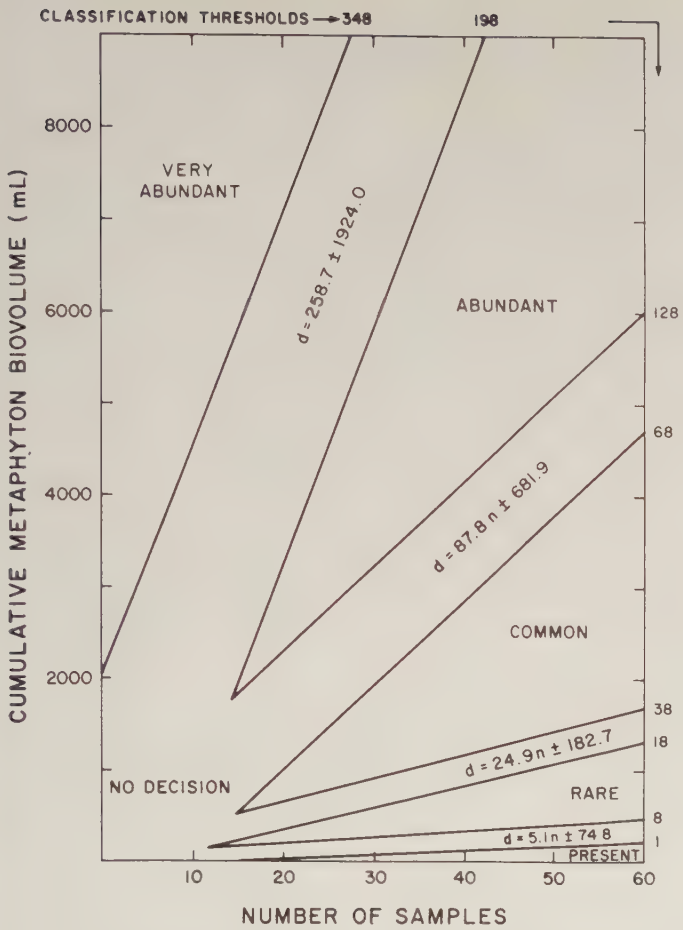

FIG. 3. Sequential sampling plan for monitoring the impact of anthropogenic acidification on the abundance of metaphytic filamentous algae in Canadian Shield lakes. Classification thresholds (average metaphyton biovolume per square metre), decision line regression equations ($\alpha = \beta = 0.05$; d = cumulative metaphyton biovolume; n = number of samples), and categorization "windows" ("Present" to "Very Abundant") are indicated.

moved along the lake. After each collection of five samples at a particular location, the cumulative total biovolume was calculated and a decision made whether to continue to another location (i.e. still within the zone of no-decision in Fig. 3) or to stop sampling (emergence out of a "window" in Fig. 3). A minimum of 20 samples (four locations) was always collected. If the cumulative total biovolume remained within the no-decision category once all the sampling locations on a particular lake had been visited, the metaphyton abundance was assigned to one of the adjacent categories based on the truncated decision method (Jackson and Resh 1988).

Macrograzer Sampling

For 30 of the study lakes, 40 modified minnow traps (enlarged openings; painted black) were set overnight around the shoreline at depths of 0.2–2.0 m in regions of rocks/boulders and macrophytes. For the remaining six lakes (Buck, Foote, Bentshoe, Myers, Galla, Gullfeather), a sampling density of 20 traps was used as in France et al. (1991). Collins et al. (1983) found that trap catches of crayfish were repeatable between years and that differences among lakes in time of trap collection following an overnight set did not affect catches. To eliminate catch bias due to size and sex dominance, only the

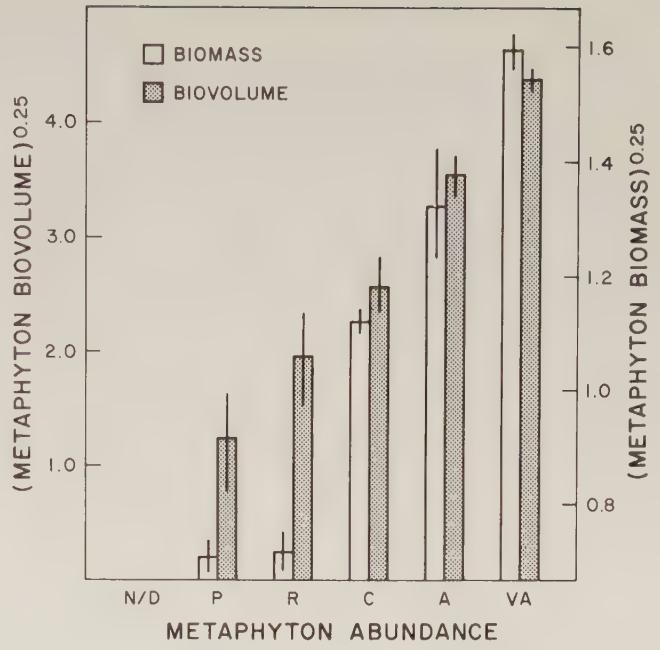

FIG. 4. Relationship between ranked metaphyton abundance from sequential sampling (N/D = "Nil"/"Detected"; P = "Present"; R = "Rare"; C = "Common"; A = "Abundant"; VA = "Very Abundant"; see Fig. 3) and lake mean biovolumes$^{0.25}$ (mL·m^{-2}, and biomasses$^{0.25}$ (mg·m^{-2}). Error bars represent ± SD.

number of adult male crayfish caught per trap was compared. Each trap was baited with three perforated plastic film canisters filled with fish-flavoured canned cat food, beef-flavoured canned dog food, and boiled macaroni. Catches of tadpoles and crayfish in Plastic and Crosson lakes did not differ from those obtained the previous year when beef liver and lettuce were used as bait (France et al. 1991). Catches of algivorous cyprinids, however, were substantially reduced compared with 1987 samplings. This was probably a result of the black paint used on the traps in the present study (N. C. Collins, University of Toronto, Toronto, Ont., unpubl. data). Because of this, all fish data unfortunately had to be excluded from analysis. Lakes were sampled between June 20 and August 4, a time at which trapability was considered to be highest (e.g. Somers and Stechey 1985; France et al. 1991) due to temperature and life cycle events (France and Welbourn 1991; R. L. France, unpubl. data).

Other Data

Water samples for pH, alkalinity, colour, Secchi depth, and total phosphorus and nitrogen concentrations were taken in the littoral zone when each lake was visited for estimation of grazer abundances and again during assessment of metaphyton development. Chemical samples were analyzed using standard OMOE procedures. Data on lake morphometry (area, perimeter length, mean depth), cottage development, and fish composition (number of algivorous or predaceous fish species) were obtained from the Ontario Ministry of Natural Resources (OMNR).

Statistical Analysis

Due to limited sample sizes (<36 in all cases), use of quantal trap catches for grazers, and the nonnormality of some of the

TABLE 2. Status of metaphytic filamentous algae in 36 acid-sensitive lakes in central Ontario. Questionnaire survey: C = Common; N = Nil. Metaphytic rank: N = Nil; D = Detected; P = Present; R = Rare; C = Common; A = Abundant; VA = Very Abundant. Biovolume and biomass are expressed as averge $mL \cdot m^{-2}$ or $mg \cdot m^{-2}$.

Lake	Questionnaire survey	Metaphyton rank	% transects	% quadrats	Biovolume	Biomass
Plastic	C	R	6/7	28.6	8.0	—
Crosson	N	N	0	0	0	0
Grindstone	N	C	4/5	32.0	59.0	1.75
Tock	C	A	4/4	0	143.4	3.92
Shoe	N	N	0	0	0	0
Heney	C	A	4/5	56.0	157.8	1.95
Clinto	C	R	2/4	20.0	15.2	0.27
Ril	C	R	4/6	46.7	18.7	0.31
Menominee	N	N	0	0	0	0
Little Leach	C	A	2/4	50.0	175.0	5.66
Wolf	C	VA	4/4	70.0	393.8	7.24
Livingstone	N	N	0	0	0	0
Dotty	N	P	1/5	8.0	13.3	0.52
Toad	C	R	4/6	20.0	6.3	0.21
Leonard	C	R	4/11	18.2	8.7	0.14
Pine	N	N	0	0	0	0
Spring	N	N	0	0	0	0
Prospect	N	N	0	0	0	0
Bear	N	N	0	0	0	0
Kimball	N	N	0	0	0	0
Crown	C	C	6/6	56.7	63.9	1.51
Rutter	C	A	4/6	26.7	169.9	3.01
High	N	N	0	0	0	0
Round	N	D	0	0	0	0
Hughes	C	N	0	0	0	0
Buck	—	R	1/6	46.6	3.1	0.24
Foote	N	N	0	0	0	0
Diamond	N	R	4/6	20.0	15.1	0.32
Sugar	N	C	2/5	24.0	54.4	1.57
Manitawaba	C	VA	6/7	71.4	313.6	5.13
Salmon	N	R	2/5	44.0	25.8	0.33
Gullfeather	—	R	2/6	20.0	12.3	0.53
Bentshoe	—	A	9/9	60.0	101.6	2.05
Galla	C	P	1/6	3.3	0.7	0.03
Myers	N	D	0	0	0	0
Little Clear	—	N	0	0	0	0

data, the nonparametric analyses of Fisher's exact probability test, the Kruskal–Wallis test, and Kendall's rank correlation coefficient were used (Siegel 1956). For those 11 lakes which were found to have metaphyton abundances of "Common," "Abundant," or "Very Abundant," Pearson's correlation coefficient was used to assess the parametric linear association between actual values for each independent variable and the fourth root-transformed (see Howell et al. 1990) metaphyton biovolumes.

Results and Discussion

Ranked designations of metaphyton abundance were closely related to lake mean biovolumes and biomasses (Fig. 4; Table 2), which themselves were strongly correlated [$(\text{biomass})^{0.025} = 0.005 + 0.38\ (\text{biovolume})^{0.25}$, $R^2 = 0.99$, $P = 0.0001$, $n = 20$]. Differences at lower metaphyton abundances (Fig. 4) reflect the proportionally greater contribution of water to the biovolume measurements. The proposed sequential sampling program therefore provides an accurate assessment of metaphyton abundance. Further, the ranked abundance categories were also closely related to both large-scale and fine-scale patterns of areal cover expressed, respectively, as either the average proportion of transects or individual quadrats per lake which were found to contain filamentous algae (Fig. 5; Table 2). Sequential sampling can therefore be used as a cost-effective technique enabling the quantitative and objective measurement of metaphyton development in lakes. For example, based on data from Howell et al. (1990) and France et al. (1991), the metaphyton rank for Plastic Lake has declined from "Abundant" in 1986 to "Common" in 1987 and to "Rare" at the sampling for the present study in 1988. Although this may reflect the general improvement in water quality occurring in Plastic Lake (Dillon et al. 1987b), similar progressive declines in metaphyton development have been subjectively noticed over this time period by cottagers on several other nearby lakes. During the summers of 1987 and 1988, this region experienced atypically prolonged periods of elevated temperatures relative to those in previous years, and this may have contributed to a retardation of zynematacean growth. Year-to-year variability in metaphyton abundance has also been reported for Adirondack lakes (P. Bukaveckas, Indiana University, unpubl. data).

There was a good agreement between the present quantification of metaphyton abundance and that of its recognition by

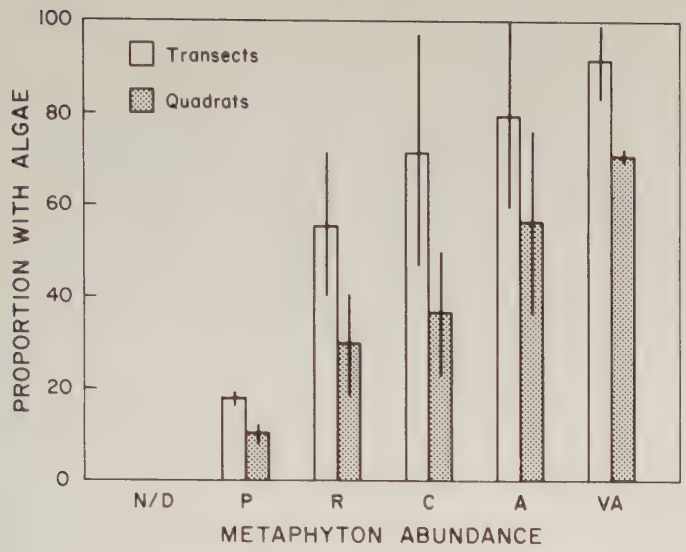

FIG. 5. Relationships between ranked metaphyton abundance (abbreviations as in Fig. 4) and the average proportion of transects and quadrats per lake which contained filamentous algae. Error bars represent ± SD.

cottagers in the questionnaire survey (SPR Assoc. Inc. 1986). Fourteen of the 18 lakes (77%) designated as "Nil" by cottagers in 1985 contained metaphyton abundance rankings of "Present" or below; large divergences between the two surveys existed for only Grindstone, Sugar, and to a lesser extent Diamond and Salmon lakes (Table 2). Cottagers were less precise in their subjective assessment of "Considerable" status; fully half of those lakes in which cottagers had stated that metaphyton (termed "filamentous algae capable of being picked up" in the questionnaire) was "Considerable" actually had abundance rankings of "Rare" or below. The strategy of a questionnaire survey network to economically gauge shifts in biological characteristics of a large number of lakes (in SPR's case, 330 lakes) seems commendable. The present results indicate that although cottagers can recognize the presence of metaphytic filamentous algae with a fair degree of accuracy, their ability to subjectively distinguish between "Some" or "Considerable" on a whole-lake basis is weak. This is no doubt a reflection of each individual's biased familiarity with the immediate area surrounding his/her cottage rather than that of the entire lake.

Metaphytic filamentous algae were present in 23 (64%) of the lakes sampled in this study: 2 "Detected," 2 "Present," 9 "Rare," 3 "Common," 5 "Abundant," and 2 "Very Abundant" (Table 2). Exploratory analysis (Wald-Wolfowitz Runs and Cox and Stuart Trend tests; not shown) suggested that lake pH, alkalinity, total nitrogen concentration, and abundance of crayfish were related to metaphyton status. More rigorous analyses showed that the environmental variables most strongly associated with metaphytic algae were pH and alkalinity (Table 3). A larger proportion of lakes characterized by low pH or alkalinity contained abundant metaphyton compared with those lakes characterized by high pH and alkalinity. Not only did acidification status clearly have a substantial influence on the presence or absence of metaphytic algae (Fig. 6A), but it also influenced the extent of metaphyton proliferation. The median pH or alkalinity of lakes in which metaphyton was rare or absent was significantly ($P < 0.05$) higher than for those lakes in which it was abundant. Ranked pH's and alkalinities were significantly ($P < 0.01$) negatively correlated with ranked metaphyton biovolumes and biomasses. Alkalinity was the highest correlate of metaphyton abundance for the subset of 11 lakes having mean biovolumes >25 $mL \cdot m^{-2}$.

Although an ordinal scale for ranking metaphyton status is useful for measuring stepwise, quantal differences in mean biovolume among lakes or within a lake through time, truly quantitative biomonitoring benefits from ascription of continuous numerical values to these catagorical divisions. In the case of metaphyton, cottager concerns reflect both the abundance and spatial cover of the nuisance algae. The Metaphyton Development Index (MDI) was therefore developed as an interval variable which integrates both these algal attributes. Each lake, based on its designated abundance rank as determined through sequential sampling, is simply weighted with the appropriate mean percentage areal cover value determined from the quadrat sites: "Nil" and "Detected" = 0%, "Present" = 11%, "Rare" = 30%, "Common" = 38%, "Abundant" = 57%, and "Very Abundant" = 71% (Fig. 4).

Calculation of the MDI for each lake showed the demonstrable effect of pH on proliferation of filamentous green algae

TABLE 3. Test of biotic and abiotic environmental determinants of metaphyton abundance in central Ontario lakes. Sample sizes are 31–36 for the nonparametric tests and 10–11 for Pearson's correlation coefficient (see text). Statistical significance indicated at $P = 0.15^*, 0.10^{**}, 0.05^{***}, 0.01^{****}$, and 0.001^{*****}.

Parameter	Fisher exact	Kruskal–Wallis	Kendall		Pearson
			Biovolume	Biomass	
pH	15.11****	10.64***	−0.33****	−0.36****	−0.27
Alkalinity	13.29***	14.18****	−0.41*****	−0.41****	−0.76****
Crayfish abundance	8.90*	5.45*	−0.22**	−0.19*	−0.03
Tadpole abundance	5.51	3.95	−0.22**	−0.29***	−0.35
Secchi depth	5.39	2.35	0.00	0.01	−0.23
TP	8.14	0.29	0.06	0.04	−0.09
TN	7.52	1.40	0.03	0.04	0.22
Colour	6.56	3.85	−0.14	−0.13	0.42
Area	5.32	1.18	0.00	0.02	−0.05
Perimeter	4.12	0.77	0.11	0.16	−0.01
Mean depth	7.11	3.42	−0.06	−0.01	−0.52**
Cottage development	3.29	0.40	−0.06	−0.10	−0.11
Predatory fish species	—	—	0.23**	0.27**	—

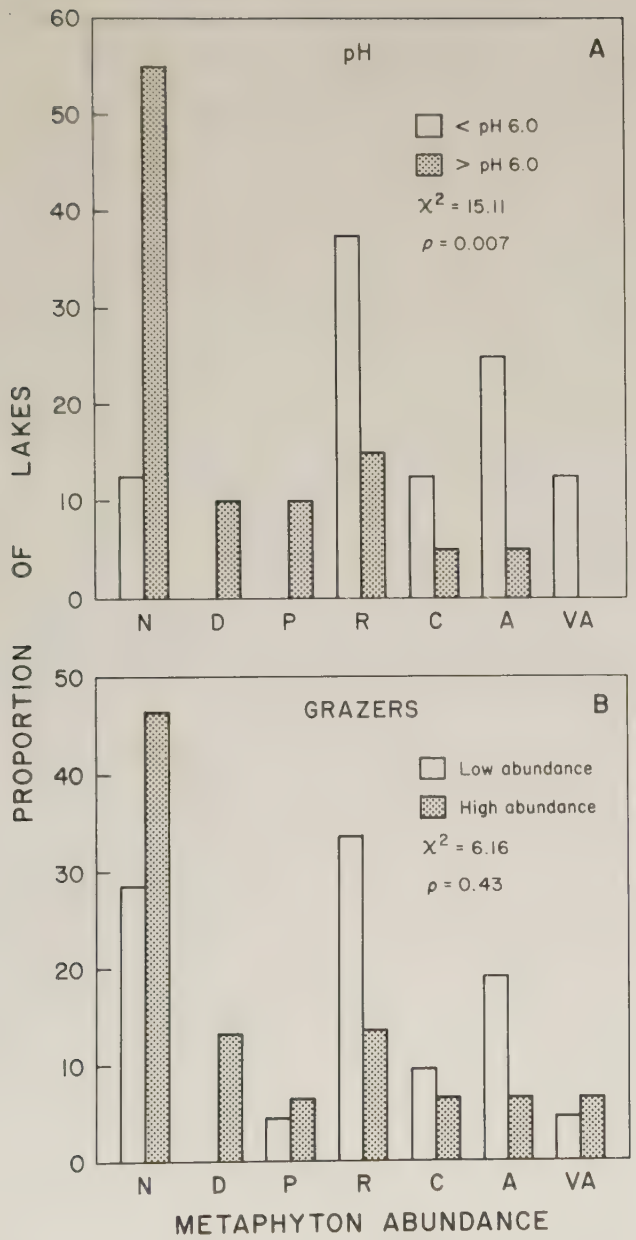

FIG. 6. Proportional distribution of ranked metaphyton abundance (abbreviations as in Fig. 4) in relation to (A) pH and (B) total grazer abundance (see text).

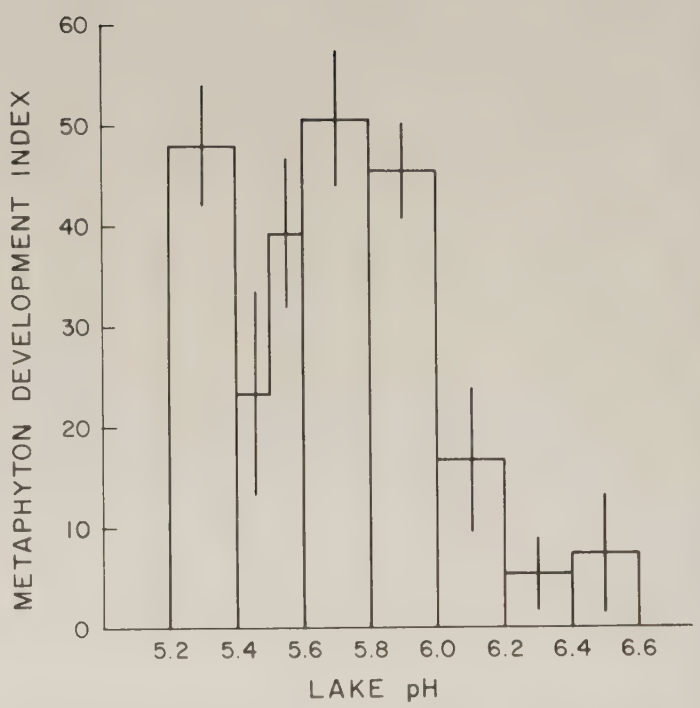

FIG. 7. Influence of lake pH on the development of metaphytic algae (see text) for all 36 lakes surveyed. Sample sizes for pH divisions were, from left to right, 2, 5/4, 2, 7, 8, 8, and 4. Error bars represent ± SD.

(Fig. 7). There is an obvious step function in the pH distribution of MDI among lakes. Although the MDI's for those lakes above pH 6 were not significantly different from one another (ANOVA, $P > 0.01$), they were much reduced ($P < 0.01$) compared with the MDI's determined for lakes less than pH 6 which in turn were also not significantly different from each other. This conclusion was based on the exclusion of one acidic lake, Crosson, which was contrary to the general pattern in that it did not contain metaphyton possibly due to having a high abundances of grazers (France et al. 1991; see next section). The fact that the development of metaphyton across the lake set displays a threshold effect of pH 6 explains why the linear correlation between metaphyton abundance and lake pH (Table 3) was not higher.

Metaphyton abundance was negatively related to the abundance of macrograzers (Table 3), although this effect was secondary to the dominating influence of lake acidification status. A marginally larger proportion ($P = 0.15$) of lakes characterized by having low numbers of crayfish (*Cambarus bartoni, Orconectes virilis, O. propinqus, C. robustus, O. immunis*) contained abundant metaphyton compared with those lakes characterized by having high crayfish catches. A similar relationship did not exist for tadpoles (*Rana clamitans, R. catesbeiana*). Abundances of macrograzers together did not significantly influence the presence or absence of metaphytic algae in the studied Ontario lakes (Fig. 6B). However, there was indication that in those lakes predisposed to metaphyton accumulation (due to, for instance, lowered pH), macrograzers did affect filamentous algal abundance. The median density of crayfish was marginally higher for those lakes in which metaphyton was rare or absent than for those lakes in which metaphyton was abundant. Rankings of both crayfish and tadpole densities were weakly but significantly ($P = 0.15$–0.05) linearly correlated with ranked metaphyton biovolumes or biomasses for all 36 lakes. Closer examination showed that macrograzer abundances (ranked through a similar sequential sampling program: France and Welbourn 1991; R. L. France, unpubl. data) had a demonstrable impact on metaphyton development for the 23 lakes which contained filamentous algae (Fig. 8). This relationship was further strengthened when two "anomolous" lakes, Manitawaba and Heney, were excluded (see below). Actual densities of crayfish or tadpoles were not, however, linearly related to metaphyton biovolume in the subset of 11 lakes containing mean biovolumes >25 $mL \cdot m^{-2}$. Clearly, lake alkalinity had a dominating influence over that of macrograzer abundance (Table 3). For example, r^2 values for the relationships between tadpoles, crayfish, and their combined densities and the ratios of metaphyton biomass per alkalinity level were −0.18, −0.22, and −0.25, respectively, but still not significant ($P > 0.10$). At best, then, macrograzers

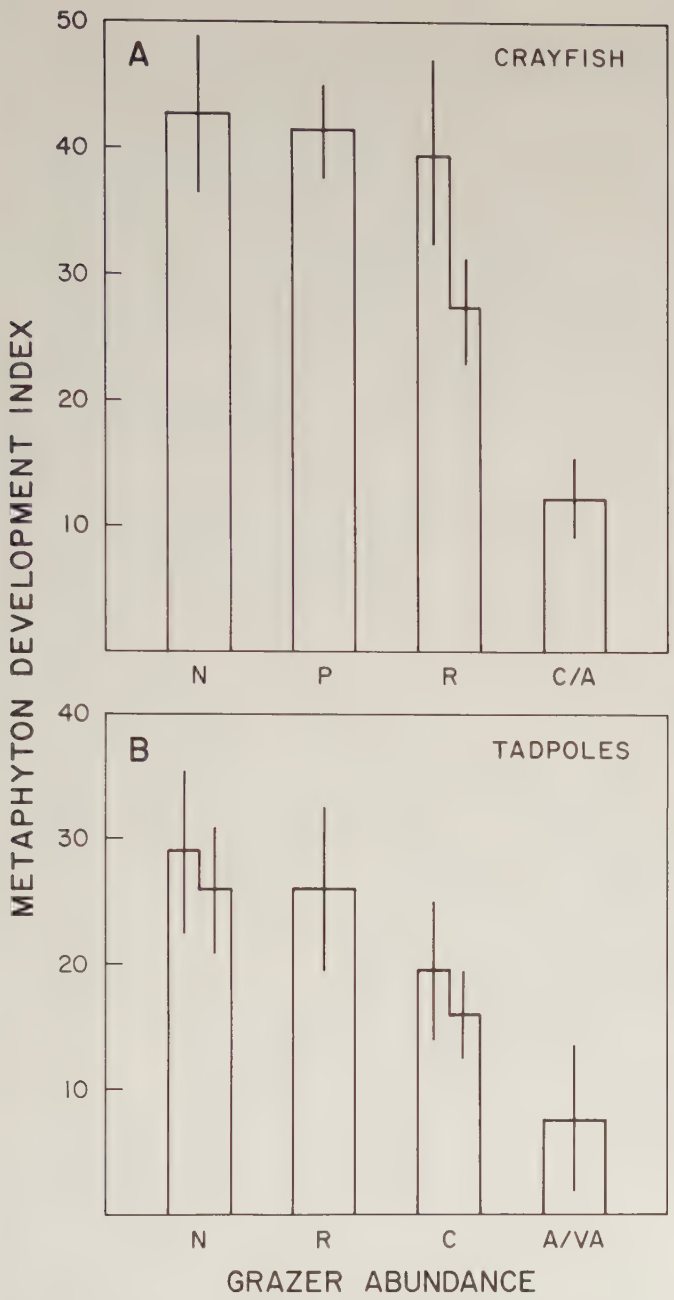

FIG. 8. Influence of ranked abundance of crayfish and tadpoles from sequential sampling programs (see text) and metaphyton development index for the 23 lakes which contained filamentous algae. Samples sizes were, from left to right, for crayfish divisions 6, 7, 6/4, and 4 and for tadpole divisions were 6/5, 8, 5/4, and 4.

only explain a fraction (no higher than 25%) of the residual variation left unexplained with relation to the dominating influence of alkalinity.

Two lakes existed which contained both prominant populations of grazers as well as "Abundant" or "Very Abundant" rankings of metaphyton: Manitawaba and Heney. The average mean depth for these two lakes, 3.8 m, was significantly less than that for the other nine lakes (7.0 m) with substantial metaphyton biovolumes. Because photosynthesis in zygnematacean algae is strongly dependent on ambient light, metaphyton distribution is negatively related to depth within an individual lake (Howell et al. 1990). It was not surprising, therefore, to find an inverse relationship between lake mean depth and metaphyton biovolume for the 11-lake subset (Table 3). Lakes like Manitawaba and Heney, because of their extensive and shallow littoral zones, may contain substantial developments of metaphyton irrespective of the abundance of macrograzers. In such cases, facultative cropping of zygnematacean primordia when they are still part of the multispecific periphyton mat may be completely overwhelmed by the optimal euphotic conditions. Once "cloud" formation begins and the algae become metaphytic, it is probably too late for macrograzers, even if present, to crop these algae (France et al. 1991).

Despite criticisms that regression procedures have little use for interpreting trophic interactions (e.g. Carpenter and Kitchell 1988; Carpenter 1988), such methods have proven successful for undertaking among-system comparisons (McQueen et al. 1990, 1992; Quiros 1990; Carpenter et al. 1991). In particular, because the majority of benthic grazing studies are short-term, small-scale experiments, there is a paucity of survey-based data with which to compare our results. Therefore, understanding whether the experimentally demonstrated potential of herbivory is actually realized in the natural environment on scales closer to those approaching equilibrium conditions is extremely limited. Of these few studies, and in support of the present findings, Creed (1988) found an inverse relationship between the presence of crayfish (*O. propinqus*) and the distribution and abundance of *Cladophora* along a reach of a Michigan stream.

The biomanipulation of grazers as a control strategy for the reduction of nuisance planktonic algae in eutrophied systems has been suggested (e.g. Benndorf 1988). Strong cases can be made for the importance of both crayfish and tadpoles in structuring littoral aufwuchs communities. Crayfish in oligotrophic lakes are opportunistic feeders, consuming when present, filamentous green algae (Capelli 1975), and can limit overall periphyton production (Flint and Goldman 1975). Specifically, crayfish are known to be capable of dramatically reducing the standing crop of macroalgae (Creed 1988) and have been associated with shifts from filamentous greens to diatoms in small manipulations (Aloi 1988). The use of crayfish for management of macrophytes, the extent of control being proportional to crayfish abundance, has been demonstrated in a number of studies (Abrahamsson 1966; Dean 1969; Lodge and Lorman 1987; Magnuson et al. 1975). Similarly, because of their close association with the benthic substrate and their dentition morphology (Gosner 1959), Ranaidae tadpoles also feed by scraping, cutting, and gorging periphyton (Heyer et al. 1975). In such cases, filamentous algae frequently constitute much of the bulk of ingested material (Gosner 1959; Jenssen 1969). Experimental manipulations have also demonstrated that green frog tadpoles are capable of substantially reducing the standing crop of filamentous green algae (Dickman 1968). The abundance of crayfish and tadpoles in the present study lakes was dependent on lake acidification status and the extent of cottage development, respectively (R. L. France, unpubl. data; France and Welbourn 1991). Stocking of acid-tolerant species of crayfish (e.g. *C. bartoni* or *C. robustus*: Berrill *et al.* 1985; Collins et al. 1981) or anurans (e.g. *R. clamitans* or *R. catesbeiana*: McDonald *et al.* 1984; Gascon and Bider 1985) in lakes with rocky littoral areas and riparian vegetation near amphibian breeding sites is worth investigating as a potential future strategy for management. For example, coincident with the experimental neutralization of Bowland Lake, Ontario (pH 4.8–5.5 increased to 6.3–6.7), metaphytic algae disappeared at which time catches of the crayfish (*C. robustus*) more than doubled (Jackson et al. 1990). Reacidification during the subsequent

year (pH 5.7–6.7) was accompanied by increased growths of metaphyton and declines in crayfish catches, both to preneutralization levels.

The number of species of predatory fishes (mostly centrachids and salmonids) was weakly but positively correlated with metaphyton abundance (Table 3). Power et al. (1985) found that the grazing rate of minnows was reduced to such an extent in the presence of predaceous fish to enable the proliferation of filamentous green algae. A similar process of cascading trophic interaction via behavioural resource depression (sensu Charnov et al. 1976) may be operating in these Ontario lakes. The foraging behaviour of both crayfish (Stein 1977; Collins et al. 1983) and amphibian larvae (Macan 1966) is retarded when they are exposed to predators. This may be an area for future attention with respect to lake management. For example, the stocking of centrachids by government agencies to satisfy immediate cottager wishes for recreational fishing might inadvertently be exacerbating metaphyton buildup in those lakes which, because of their acid sensitivities and shallow littoral zones, are already predisposed to this problem.

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LETTERS AND COMMENTS
LETTRES ET COMMENTAIRES

Comment on Gauldie (1991)

In a recent perspective in the *Journal*, Gauldie (1991) follows a thoughtful analysis of the historical basis of the stock concept in fisheries with a seriously flawed critique of the use of molecular genetic approaches to examining population structure in fishes. Gauldie evidently views such studies as a waste of time and resources. Gauldie's arguments in this context rest on two general assertions: (1) that most such studies have failed to detect genetically differentiated stocks and (2) that cases where genetic variation among localities has been observed are the product of selection acting on alternate alleles, and not stochastic influences in reproductively isolated populations.

Although Gauldie's views are extreme, they have the virtue of being at least partially supported by the facts in the case of protein polymorphism. Most protein electrophoretic studies have indeed failed to detect genetically differentiated stocks in marine species (e.g. Smith et al. 1990), and some protein polymorphisms are almost certainly maintained by selection. Gauldie is wrong, however, to dismiss stochastic influences as a principal factor in the maintenance of most genetic variation. Moreover, his arguments fall particularly far off the mark in the case of studies of mitochondrial DNA (mtDNA) polymorphism.

Gauldie argues energetically against the use of protein electrophoresis in population surveys, but he saves his strongest salvos for mtDNA studies. In Gauldie's words, "The process of divorcing genetics from biology reaches its nadir in the use of mitochondrial DNA in stock separation studies." In fact, Gauldie's comments on the subject are most notable for the extent to which they are divorced from the facts concerning mtDNA analysis and its application to fisheries research. My major purpose in this comment is to correct Gauldie's errors regarding mtDNA. Interested readers should consult reviews by Avise et al. (1987) and Moritz et al. (1987) for more information.

Gauldie first attacks the use of restriction endonucleases to assay genetic variation in mtDNA. His oversimplified description of how the method works is followed by the critique that "The only possible rationale for using bits of DNA is that the order of codons is less significant than the juxtaposition of codons. This seems to be an unlikely prospect." This criticism is cryptic, since the distinction between "order" and "juxtaposition" of codons is undefined. In any case, the criticism is unjustified, since the order of codons is not at issue in population studies of mtDNA polymorphism. By far the greatest part of the intraspecific sequence variation in mtDNA detected by restriction endonucleases either produces only synonymous changes in codons or else occurs in sequences that do not contain codons at all. Codon order is not affected.

Gauldie's second criticism states that some mitochondrial proteins are polymorphic, and given their important physiological role, that selection is likely to be important on them, and hence on the DNA that codes for them. This is true, but is of virtually no significance to studies of intraspecific mtDNA polymorphism for two reasons. First, the vast majority of mitochondrial proteins are not coded by mtDNA, but by nuclear DNA. mtDNA codes for 13 proteins, 12 of which are subunits of larger enzyme complexes; by contrast, hundreds of proteins are involved in mitochondrial function as well as replication and processing of mtDNA and the products that it codes for (Brown 1985). Second, numerous studies have shown that the great majority of mtDNA polymorphisms detected within species (indeed, even among closely related species) are silent, that is, they have no effect on the amino acid composition or function of the proteins encoded by the mtDNA (e.g. Carr and Marshall 1991).

Third, Gauldie asserts (correctly) that mtDNA is functionally haploid and that haploid populations go to fixation more rapidly than diploid population. His conclusion, however, that "information. . . contained in the mitochondrial genome. . . is therefore of little value in any biological context outside of the mitochondrion itself" is wrong. As Birky et al. (1983, 1989) have shown, the haploid nature and maternal inheritance of mtDNA lead to the expectation (given equal numbers of males and females, and equal migration of the sexes) that mtDNA will show about four times the degree of genetic differentiation among populations linked by some degree of gene flow than will nuclear genes or gene products. In practical terms this means that subtle differences among populations are easier to detect in mtDNA than in nuclear genes, a point supported empirically by a number of cases where both allozymes and mtDNA have been studied (e.g. Avise et al. 1986). Given this extra sensitivity of mtDNA analysis, mtDNA surveys may indicate population divisions where surveys of nuclear gene products do not, an advantage, not a short-coming of mtDNA analysis. As Gauldie notes correctly in another context, the amount of migration required to homogenize neutral genetic variation across populations or geographic locales is extremely small. The roughly fourfold greater migration permitted for mtDNA still implies a very high degree of reproductive isolation, the major concern for fisheries scientists attempting to delineate populations.

Finally, Gauldie suggests that mtDNA polymorphism is related to longevity, and that polymorphism accumulates over the life span of an individual, thus (presumably) making the analysis of polymorphism as an attribute of populations inappropriate. Although some mutations may indeed accumulate with age, the criticism is once again misguided. Age-related mutations such as have been detected in humans would characteristically involve somatic, not germline, tissues, and hence could not be compounded by transmission from one generation to another. Such mutations would likely involve only minor (probably single base) changes in the mtDNA sequence, and would almost necessarily affect only a small proportion of the huge number of mtDNA molecules present in an individual fish or tissue of a fish. Routine methods of screening mtDNA samples for polymorphism would be very unlikely to detect either

the mutational changes or the small proportion of molecules affected by the changes. Moreover, if mutations occurring within the life span of individuals were detected, they would manifest themselves as heteroplasmy, the condition in which two or more mtDNA genotypes coexist within the same individual. In fact, heteroplasmy is notable for its rarity (Bentzen et al. 1988); the great majority of individuals of most vertebrate species are homoplasmic. Gauldie cites sturgeon (Brown et al. 1988) as an example of a long-lived species with extensive polymorphism. Sturgeon do exhibit considerable polymorphism and heteroplasmy, but the type of polymorphism involved, length variation in the mtDNA molecule, is of a completely different sort than the restriction site variation observed in most species. Moreover, numerous examples exist of species that have much shorter life spans than sturgeon but are more polymorphic in their mtDNAs (e.g. menhaden, Bowen and Avise 1990; mummichog, Powers et al. 1986).

Gauldie's blanket characterization of genetically isolated stocks as "nonexistent" ignores many examples to the contrary, including several recent mtDNA studies that have demonstrated the existence of genetically distinct groups (i.e. "stocks") within various marine and anadromous species (e.g. Bernatchez and Dodson 1990; Bentzen et al. 1989; Bowen and Avise 1990; Chapman 1990; Dahle 1991; Carr and Marshall 1991; see also Ovenden 1990). In some cases, mtDNA studies of broadly distributed marine species have revealed striking genetic discontinuities that were previously unexpected (Saunders et al. 1986; Reeb and Avise 1990).

Gauldie's dismissal of population genetic surveys as being "biologically sterile" is unsound. I suggest that such studies, particularly those that target mitochondrial or nuclear DNA, will continue to teach us a great deal regarding the population biology and evolutionary genetics of marine species — Paul Bentzen, *Marine Gene Probe Laboratory, Biology Department, Dalhousie University, Halifax, N.S. B3H 4J1, Canada.* (JB062)

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Reply to Comment by P. Bentzen

I must dispel Dr. Bentzen's impression that my selectionist views are extreme, and therefore, within the social canons of biology, suspect, and that his neutralist views are moderate, and therefore credible. My review was aimed at the history of failure of establishing genetically isolated stocks, not as a review of neutralist theory. However, neutralist theory lies at the heart of the present problems associated with genetically isolated stocks.

I believe that there has been no more extreme and ill-founded dogma in biology than that of selectively neutral genes. The very basis of the neutralist argument, that allele frequencies distributions are a function of effective population size, automatically discounts any selection effects at the individual level in favour of stochastic effects at the population level. Apart from the group-selection problems inherent in the effective population size argument, biologists have showed over and over again in all kinds of organisms, even fishes (Mitton and Lewis 1989), that allelic proteins are very likely to be maintained at their observed frequencies by selection. Neutralist views of population genetics have proved to be so distant from experimental reality that they have been characterized by Cain (1983) as "Textbook myths. . . asserted on no direct evidence whatever, supported by a display of mathematical theorizing so simplistic that it could not possibly apply to real organisms, and protected from exposure by an avoidance of any consideration of the probable biology of the variants. . ." The selection versus neutralist issue is central to the idea of genetically isolated stocks. Any biological character whose distribution is controlled by selection cannot be used in the identification of genetically isolated stocks. Although Dr. Bentzen seems to be aware of this constraint, he still persists in viewing neutralist processes as being the most significant processes in the geographic

distribution of genes. Perhaps the most convincing demonstration of selection effects versus neutrality in fishes comes from comparing the observations of Smith who showed in one species very large instantaneous changes in frequency with the temperature (Smith 1979), and in another species, changes in gene frequency ascribed to a severe reduction in population size (i.e. effective population size) that were so small that they could only be detected by statistical means (Smith et al. 1991). I believe that in both theory and practice the idea of genetically isolated stocks of fish is ill-founded.

However, as Dr. Bentzen correctly observes, my own exasperation with the neutralist stock argument reaches its peak with the use of mitochondrial DNA. Dr. Bentzen argues that my case against the continuing use of mitochondrial DNA in the stock argument is fatally flawed on not just one, but four counts.

The first flaw arises from my "cryptic" critique of the basic methodology. Let me expand my critique. Consider the reduction of the DNA to its basic nucleotides. I think that it is self-evident that just the relative proportions of nucleotides would have by themselves almost no biological information content. Nor would, say, the proportion of juxtaposed dinucleotides. The only orderings of nucleotides that have any biological information content are those that code for genes (in the broadest sense of genes). The issue of biological information content is essential to any scientific argument. The testability of an hypothesis depends on some demonstration of cause and effect, which, in turn, requires some demonstrable, i.e. biological information containing, effect. Subsets of a gene sequence could only be used in hypothesis testing if they could be identified with particular parts of protein (or other gene product) that they code for, and if the biochemical functions of those particular parts of the protein were experimentally accessible. The alternative to causal relationships is to use tests that compare one curve (or frequency distribution) with another, a procedure that is, at best, ambivalent (Feller 1968).

Now consider the DNA sequence broken into a number of pieces by enzymes. Since these collections of bits contain no biological information that is accessible to experiments, they can only have statistical properties. I see this as an undesirable situation, reducing the scientifically testable biological information content of the genome to the point where the only possible test on an hypothesis would be by matching observed frequencies with those predicted from statistical models.

The second flaw arises from my expectation that the proteins encoded by the mitochondrial DNA are either demonstrably under selective constraints, or are likely to be so. I am sure that Dr. Bentzen is well aware of the dogma of the 1960's that most of the genome was not specifically associated with coded gene products. I would expect that few biochemists of the 1990's would agree with the idea that much of the genome was not associated with coded gene products accessible to selection. What we know experimentally of mitochondrial proteins supports the idea that most, if not all, of the "mitochondrial" genome (be it in the mitochondrion or in the cell nucleus) is accessible to, and influenced by, the agencies of selection. Unfortunately my view is almost impossible to disprove. Attempts to demonstrate "silent sequences" can easily be dismissed as in vitro artefacts that could have been overthrown by the "next test" that the experimenter had failed to provide. I do not think that arguments for "silent sequences" can be disentangled from the confounding effects of ignorance without direct experimental evidence. As biologists, we have a long history of interpreting our ignorance of a biological character as evidence of it having no significant function (Mayr 1970). Against that history and the more recent debacle of incorrectly assigned neutrality of protein alleles, it is very brave of Dr. Bentzen to hold out for selectively neutral DNA sequences in mitochondria.

The third flaw rises from my argument for fixation trajectories being different in the mitochondrial than in the nuclear DNA. The difference in fixation rate between haploid and diploid systems is unavoidable, but some neutral theorists have taken a very elegant path to solve this problem. The essential requirements of all the neutralist models is that the "effective population size" controls the probability that a mutant is lost by stochastic errors. Consequently, the neutral theorist's task is to establish the compatibility of the effective population size of mitochondria with the effective population size of the carrier organism. The approach taken is to assume that all of the copies of the mitochondria in the individual are the same, and can therefore be treated as a single haploid entity equivalent to that of the individual genome. If further adjustments are made in terms of relative migratory behaviour of males and females, and the higher apparent mutation rates of mitochondria (they have no DNA repair mechanism; therefore, apparent rates are higher than in the nuclear DNA), then the mitochondrial effective population size can be made compatible with that of the individual carrier population.

However, mitochondria are not all equivalent copies because of their high rate of mutation. Furthermore, their generation time is much shorter than that of the individual carrier. In effect, a small population of individual carriers contains several (with respect to mutant lives) extremely large populations of mitochondria that undergo many generations within one generation of the carrier organism. The difference in effective population sizes over sampling period is therefore enormous, and, coupled with the inherently more rapid rate to fixation of haploids, makes it unlikely that mitochondrial fixation trajectories could provide information relevant to those of the carrier genome.

This difficult situation applies only in the case of assumed neutrality and vanishes when the contribution of the nuclear-DNA-coded material is taken into account. For example, it can be assumed that the fixation probabilities of mixed mitochondrial and nuclear originating DNA's are the same as the most probable of the fixation rates of either set of materials. If the nuclear originating mitochondrial protein is under selective control, as Dr. Bentzen allows, then the probability of fixation of the mixed DNA's must be 1.

The fourth, and final, flaw according to Dr. Bentzen is that in spite of the direct evidence for age-related accumulation of mitochondrial DNA mutations — in organisms ranging from *Drosophila*, through mice, to humans — it does not occur in fish. Dr. Bentzen argues that there are observations in the literature of old fish with little mitochondrial DNA variation, and vice versa. This is not so. Papers quoted by Dr. Bentzen dealing with mitochondrial DNA in fishes do not give enough information about sample age, or even size distribution, to test the general observation that those fishes that we know to generally have younger ages have less mitochondrial DNA variation than in those species that we know to have generally older ages.

Dr. Bentzen finishes by decrying my blanket condemnation of stock studies by quoting papers that he claims clearly establish stocks by mitochondrial DNA genetic variant frequencies. Perhaps Dr. Bentzen has forgotten that long, long list of stocks established just as clearly by allelic protein variant frequencies. All of those stocks vanished when the selective neutrality of

the underlying genetic variation could no longer be assumed. Even with mitochondrial DNA studies, Powers et al. (1986) (which I recommend be read alongside those papers urged on the reader by Dr. Bentzen) pointed out that selection could just as well account for the distribution of mitochondrial DNA variants over space as stochastic processes. Perhaps even more interestingly, some authors find that the neutral mitochondrial DNA variant stocks differ from stocks based on neutral protein allele variants (Reeb and Avise 1990).

The idea of genetically isolated stocks based on neutral protein allele variants could be tested, and dismissed, only because allelic proteins have identifiable functions that can be evaluated for physiologically significant changes along potential selective gradients. The neutralist support for protein allele stocks came from matching observed protein allele frequencies with those predicated from neutral theory models. Those matching distributions proved to be unconvincing in the face of direct experimental evidence. Mitochondrial DNA studies examine frequency distributions of bits of DNA whose biological information content is so low that it is unlikely that they could ever be tested experimentally along potential selective gradients. The only tests left are either to look at matches between observed mitochondrial DNA frequencies and those predicted by neutral theorists; or to use our common sense.

My choice is common sense, apparently something of an extreme choice amongst biologists these days — R. W. Gauldie, *Hawaii Institute of Geophysics, School of Ocean and Earth Sciences and Technology, University of Hawaii, Honolulu, HI 96822, USA.* (JB171)

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Guide for Authors

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together. The paper should not be submitted or accepted for publication elsewhere.

The *Journal* considers the following types of contributions:

Articles: Studies of broad scope that are original contributions to science.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science of concern to the professional and layperson. References are desirable; informal style is welcome.

Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript Submission

Submit manuscripts in triplicate to the Editor, accompanied by a covering letter that (1) states the main points and significance of the work, (2) accepts the mandatory $44 (plus 7% GST) page charge, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

Editorial Practices

The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed. 1991. Spec. Publ. No. 20, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990. Trans. Am. Fish. Soc. 119: 2–15). All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty reprints are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent with the proofs).

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 21.5 × 28 cm (8.5 × 11 in.), on only one side of the page. Leave all margins at least 2.5 cm wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and

figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as g $C/m^2/d$; use g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when page proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints of figures, or originals if they do not exceed 21.5 × 28 cm, on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as ". . . is discussed" or ". . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting

and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . ." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1½-, or 2-column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions or the original artwork (no larger than 21.5 × 28 cm) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures should be avoided if possible, as they usually reproduce poorly. Dot-matrix printers cannot produce sufficiently dark, solid lines to ensure legibility. Figures drafted in black india ink are always preferable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We prefer

to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetres per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	M
mole	mol
moles per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. comm."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)". Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

PETERMAN, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

GORDON, D. C. JR., AND A. S. HOURSTON [ED.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LEBLOND, P. H., AND L. A. MYSAK. 1978. Waves in the ocean. Elsevier, New York, NY. 602 p.

Part of book

HEALEY, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia, p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard

methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

KUTTY, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, Univ. Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

SMITH, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sousjacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans une style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata, et accompagnés d'une lettre mentionnant : 1) les principaux points et l'importance du travail, 2) l'acceptation des frais de publication obligatoires de 44 $ (plus 7 % TPS) par page, 3) des suggestions quant aux arbitres éventuels et 4) une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5e éd. 1991. Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee *et al.* (1990. Trans. Am. Fish. Soc. 119 : 2–15). Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, no 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Cinquante tirés à part sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication (un bon de commande à cet affet accompagne les épreuves).

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 21,5 sur 28 cm (8,5 sur 11 pouces), sur un côté de la page. Laissez toujours une marge d'au moins 2,5 cm.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun droit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé donne-t-il l'essentiel des connaissances nouvelles ? L'introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, date, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux, et le teste, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que g $C/m^2/d$; utilisez plutôt g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Département d'économique, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al.*

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies des figures, ou encore les originaux, s'ils ne dépassent pas 21,5 sur 38 cm. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le

défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que «Sur» ou «Vers», ou des expressions telles que «Contribution à . . .» ou «Recherche sur . . .». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que «. . . on y analyse» ou «. . . a été trouvé» ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail.

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375-500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : «Afin de déterminer . . . nous . . .». Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possibles, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendres devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½- ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit «Tableau — près d'ici».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies ou les dessins originaux (pourvu qu'ils ne dépassent pas 21,5 sur 28 cm) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1½- ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge «Fig. — près d'ici».

On devra autant que possible éviter les illustrations préparées par ordinateur, qui ne se reproduisent pas très bien. Les imprimantes par points ne peuvent produire de lignes solides suffisamment foncées pour être lisibles. On obtiendra toujours de meilleurs résultats en utilisant de l'encre de Chine noire. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètres par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
électronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	M
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : «Brown (1983) compare . . » ou «. . . on a comparé (Brown 1983)». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de «*et al.*» : «Brown *et al.* (1983) comparent . . .» ou «. . . on a comparé (Brown *et al.* 1983)».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de «comm. pers.».

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-le dans la liste des références, avec la mention «(Sous presse)». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention «données inédites».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D. ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctique. Can. J. Fish. Aquat. Sci. 40 : 1278–1292.

Numéro complet du journal

GORDON, D. C. JR., AND A. S. HOURSTON [ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa (Ont.). 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1982. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.] La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Thèse

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Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles «h,» «min» et «s» ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12:30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, «cold water» (n.) «cold-water» (adj.); ‹hard water› (n.), «hardwater› (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

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Volume 49, No. 1, January 1992

Volume 49, nº 1, janvier 1992

Arnason, A. N., M. H. Papst, and G. E. Hopky. Modelling the increase in variance of fish weight 2–16

Miskimmin, B. M., J. W. M. Rudd, and C. A. Kelly. Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water 17–22

Boudreau, P. R. Acoustic observations of patterns of aggregation in haddock (*Melanogrammus aeglefinus*) and their significance to production and catch 23–31

Simard, Y., P. Legendre, G. Lavoie, and D. Marcotte. Mapping, estimating biomass, and optimizing sampling programs for spatially autocorrelated data: case study of the northern shrimp (*Pandalus borealis*) 32–45

Tremblay, G. et L. A. Giguère. Relation longueur/écaille allométrique chez le saumon atlantique (*Salmo salar*) durant la phase marine 46–51

Grapentine, L. C., and D. M. Rosenberg. Responses of the freshwater amphipod *Hyalella azteca* to environmental acidification 52–64

Renberg, I., and H. Hultberg. A paleolimnological assessment of acidification and liming effects on diatom assemblages in a Swedish lake 65–72

Flinkman, J., I. Vuorinen, and E. Aro. Planktivorous Baltic herring (*Clupea harengus*) prey selectively on reproducing copepods and cladocerans 73–77

Whitehead, H., S. Waters, and T. Lyrholm. Population structure of female and immature sperm whales (*Physeter macrocephalus*) off the Galápagos Islands 78–84

Douglas, D. J., and S. S. Bates. Production of domoic acid, a neurotoxic amino acid, by an axenic culture of the marine diatom *Nitzschia pungens* f. *multiseries* Hasle 85–90

Wildish, D. J., A. J. Wilson, and B. Frost. Benthic boundary layer macrofauna of Browns Banks, northwest Atlantic, as potential prey of juvenile benthic fish 91–98

Foote, C. J., C. C. Wood, W. C. Clarke, and J. Blackburn. Circannual cycle of seawater adaptability in *Oncorhynchus nerka*: genetic differences between sympatric sockeye salmon and kokanee 99–109

Hinterleitner, S., M. Huber, R. Lackner, and W. Wieser. Systemic and enzymatic responses to endurance training in two cyprinid species with different life styles (Teleostei: Cyprinidae) 110–115

Kingston, J. C., H. J. B. Birks, A. J. Uutala, B. F. Cumming, and J. P. Smol. Assessing trends in fishery resources and lake water aluminum from paleolimnological analyses of siliceous algae 116–127

Cumming, B. F., J. P. Smol, J. C. Kingston, D. F. Charles, H. J. B. Birks, K. E. Camburn, S. S. Dixit, A. J. Uutala, and A. R. Selle. How much acidification has occurred in Adirondack region lakes (New York, USA) since preindustrial times? 128–141

Benton, M. J., and S. I. Guttman. Allozyme genotype and differential resistance to mercury pollution in the caddisfly, *Nectopsyche albida*. I. Single-locus genotypes 142–146

Benton, M. J., and S. I. Guttman. Allozyme genotype and differential resistance to mercury pollution in the caddisfly, *Nectopsyche albida*. II. Multilocus genotypes 147–149

Ramcharan, C. W., D. K. Padilla, and S. I. Dodson. A multivariate model for predicting population fluctuations of *Dreissena polymorpha* in North American lakes 150–158

Ramcharan, C. W., S. I. Dodson, and J. Lee. Predation risk, prey behavior, and feeding rate in *Daphnia pulex* 159–165

Lindesjöö, E., and J. Thulin. A skeletal deformity of northern pike (*Esox lucius*) related to pulp mill effluents 166–172

Bodensteiner, L. R., and W. M. Lewis. Role of temperature, dissolved oxygen, and backwaters in the winter survival of freshwater drum (*Aplodinotus grunniens*) in the Mississippi River 173–184

France, R. L., and P. M. Welbourn. Influence of lake pH and macrograzers on the distribution and abundance of nuisance metaphytic algae in Ontario, Canada 185–195

Letters and Comments/Lettres et commentaires

Bentzen, P. Comment on Gauldie (1991) 196–197

Gauldie, R. W. Reply to comment by P. Bentzen 197–199

Guide for Authors/Guide des auteurs i–x

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